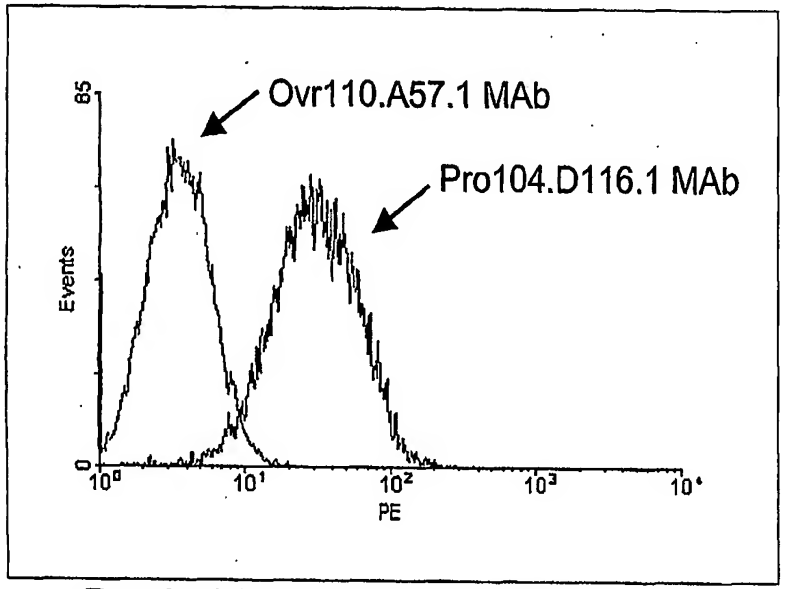
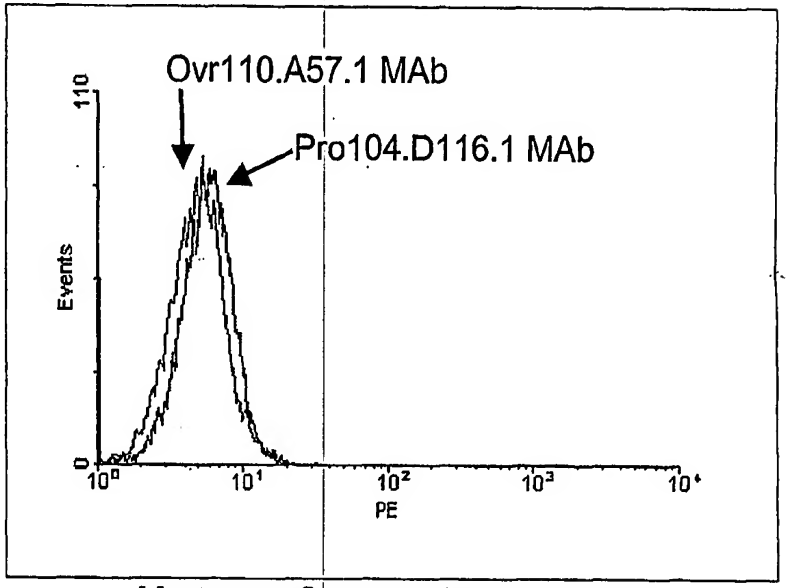
FIGURE 1. Pro104.D116.1 MAb Binds to 293F Cells Transiently Transfected with Pro104

Fig. 1A



Pro104-Transfected 293F Cells

Fig. 1B

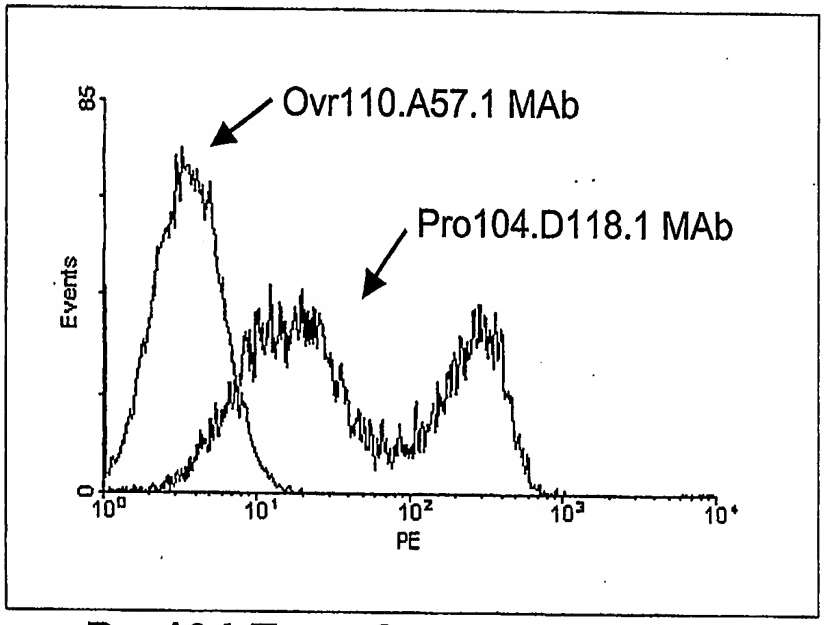


Untransfected 293F Cells

No Fill: Pro104.D116.1 MAb
Shaded: Ovr110.A57.1 Control MAb

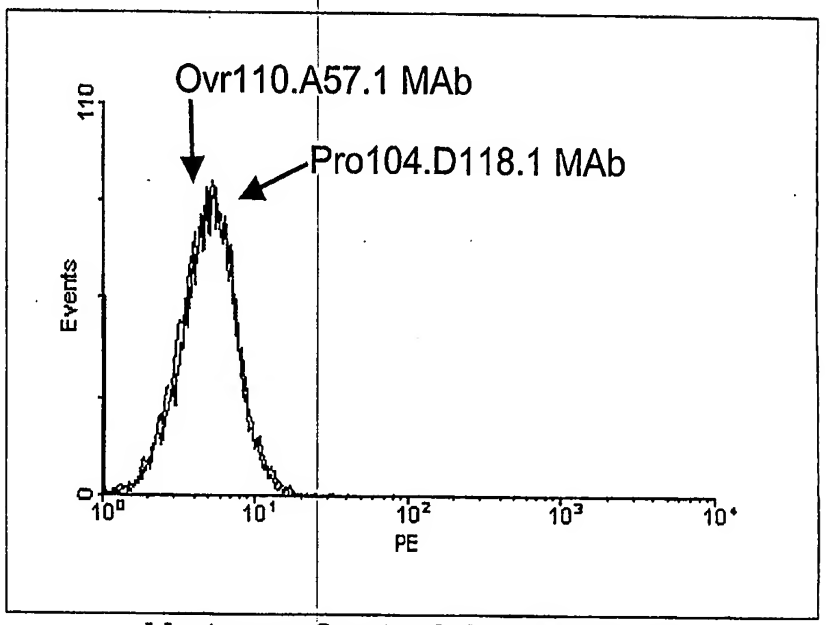
FIGURE 2. Pro104.D118.1 MAb Binds to 293F Cells Transiently Transfected with Pro104

Fig. 2A



Pro104-Transfected 293F Cells

Fig. 2B



Untransfected 293F Cells

No Fill: Pro104.D118.1 MAb
Shaded: Ovr110.A57.1 Control MAb

FIGURE 3. Pro104.C19.1 binds to live HeLa Cancer Cells Expressing Pro104

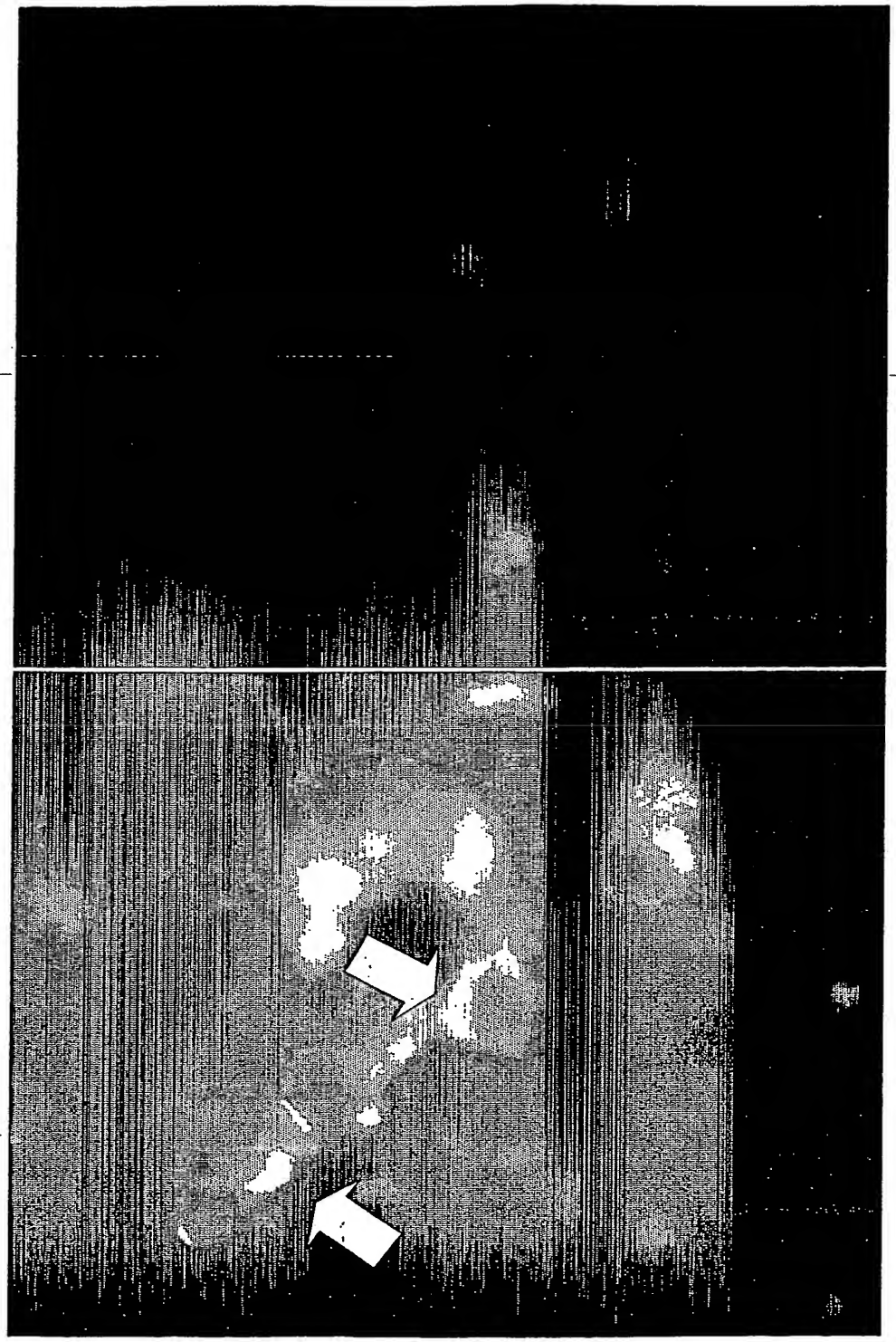


Fig. 3A. HeLa Cells Fig. 3B. SKOV-3 cells

N= Nuclei

FIGURE 4. Cy3-Pro104.C25.1 binds to live HeLa Cancer Cells Expressing Pro104

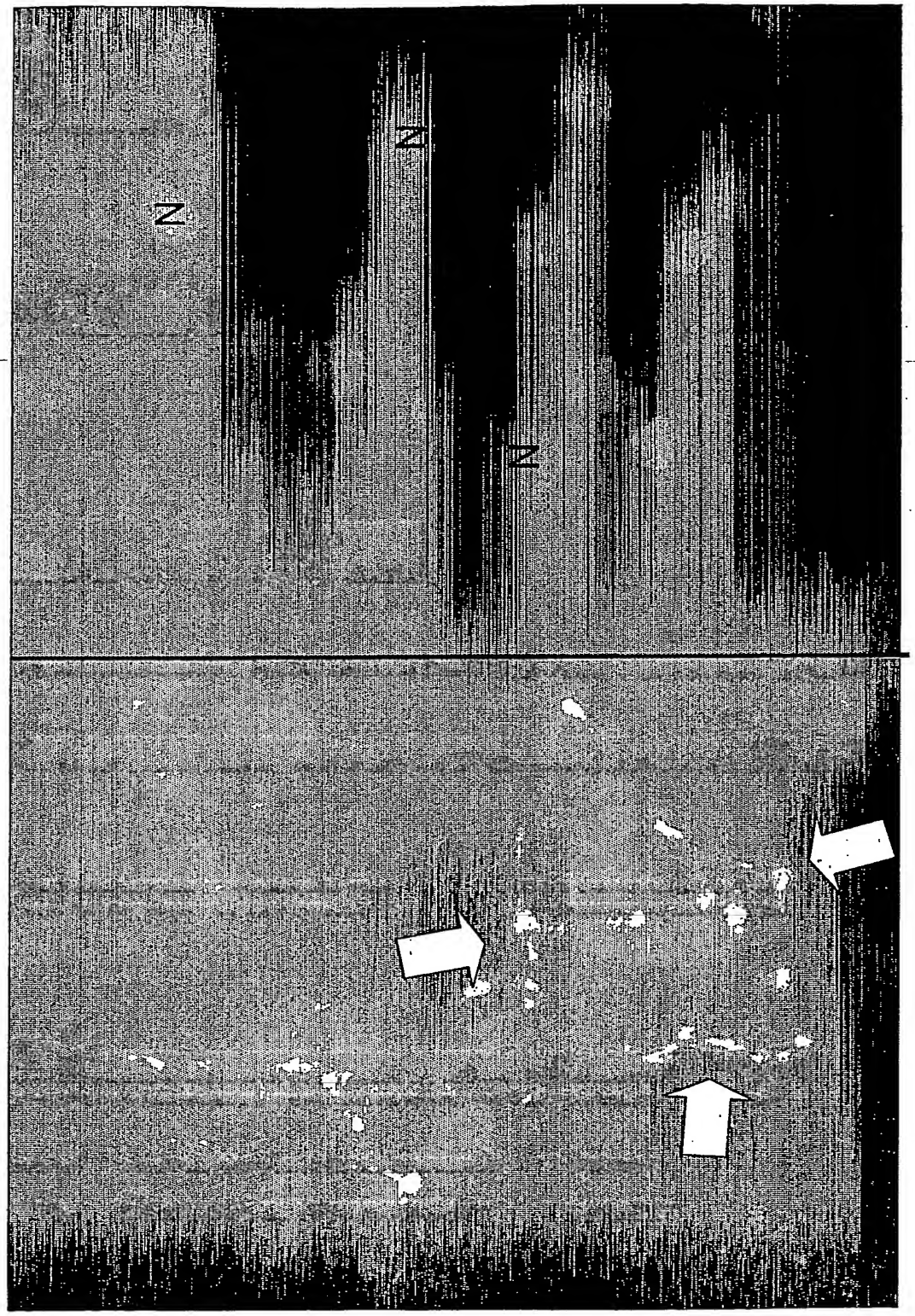


Fig. 4A. HeLa Cells Fig. 4B. SKOV-3 cells

N= Nuclei

FIGURE 5. Cy3-Pro104.C25.1 binds to and is Internalized in live HeLa Cancer Cells Expressing Pro104

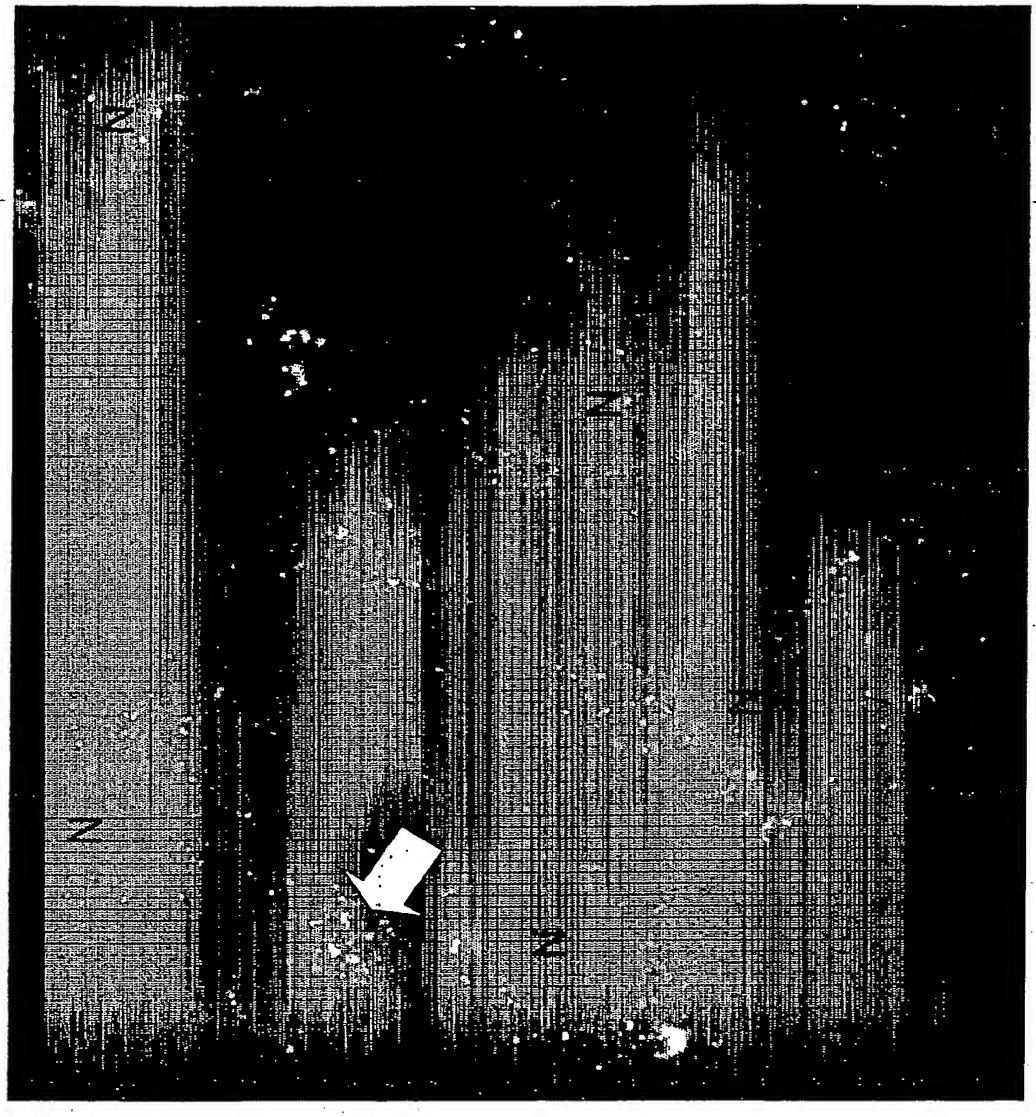


FIGURE 6. Cy3-Pro104.C19.1 binds to and is Internalized in Pancreatic Cancer Cells Expressing Pro104

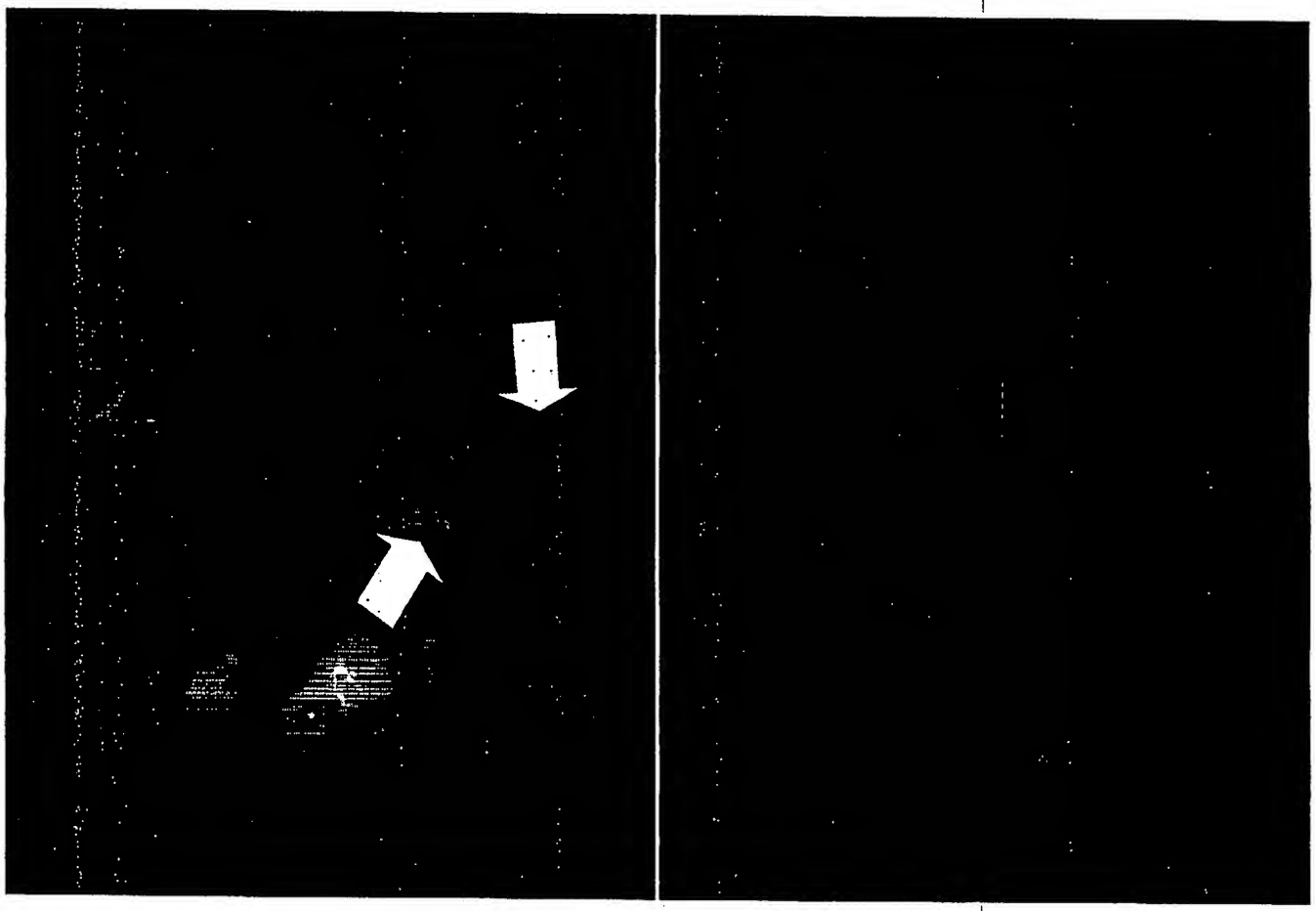


Fig. 6A. MIA PaCa-2 Cells

Fig. 6B. HCT-116 cells

N= Nuclei

FIGURE 7. Cy3-Pro104.C55.1 binds to and is Internalized in Pancreatic Cancer Cells Expressing Pro104

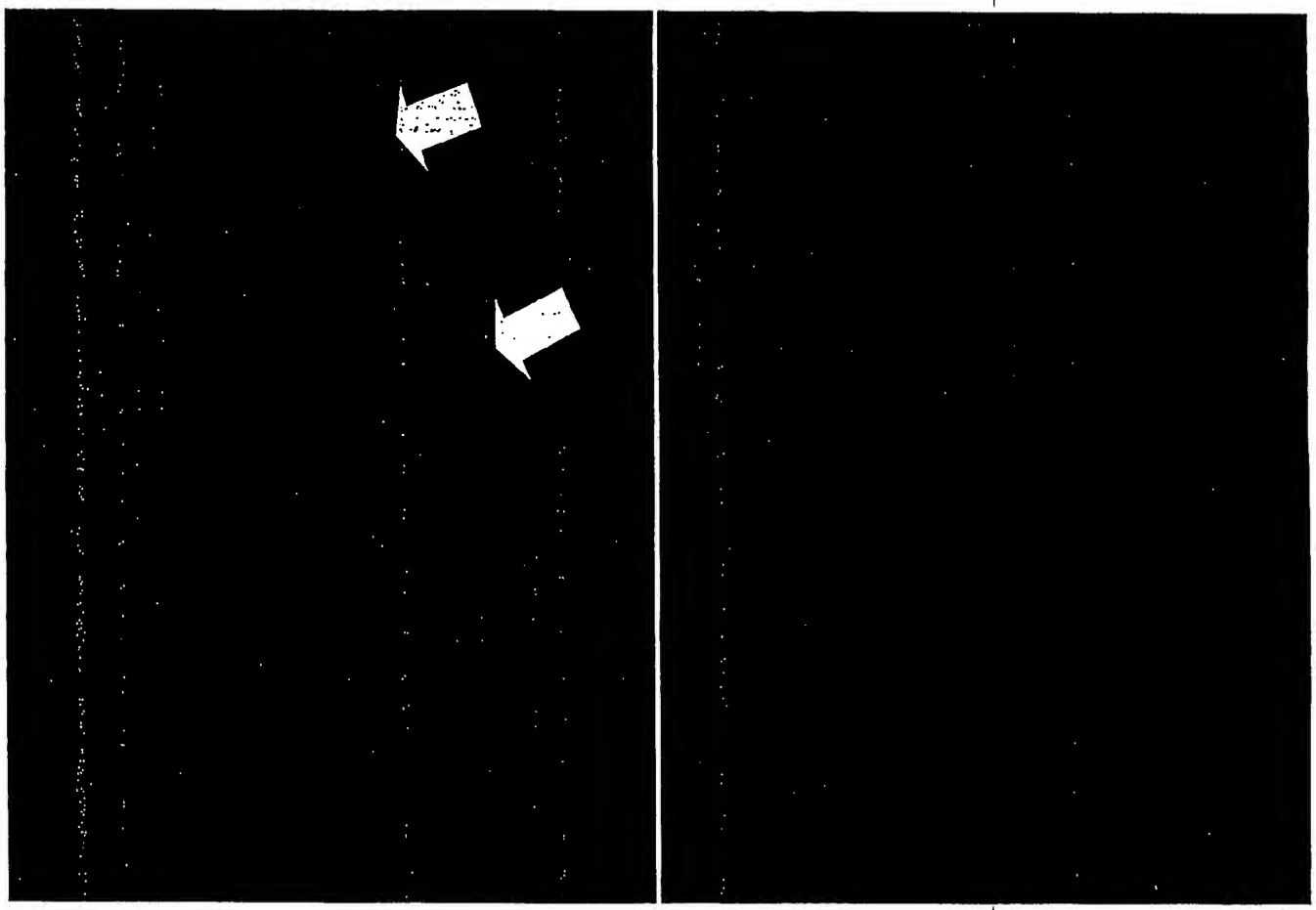


Fig. 7A. MIA PaCa-2 Cells

Fig. 7B. HCT-116 cells

N= Nuclei

FIGURE 8. Pro104.C25.1 binds to Pro104 on Cancer Cells in Ovarian Tumors

Fig. 8B normal ovary 1

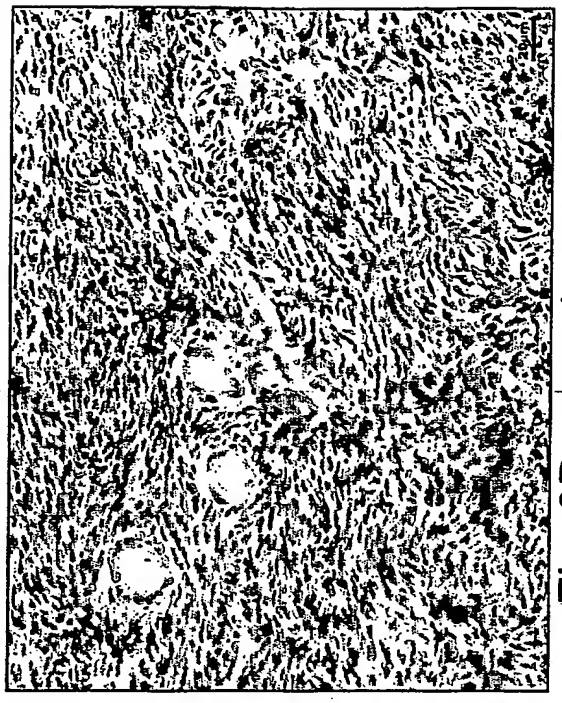


Fig. 8D normal ovary 2

Fig. 8A ovarian cancer 1

Fig. 8C ovarian cancer 2

FIGURE 9. Pro104.C25.1 binds to Pro104 on the Cell Membrane of Ovarian Cancer Cells

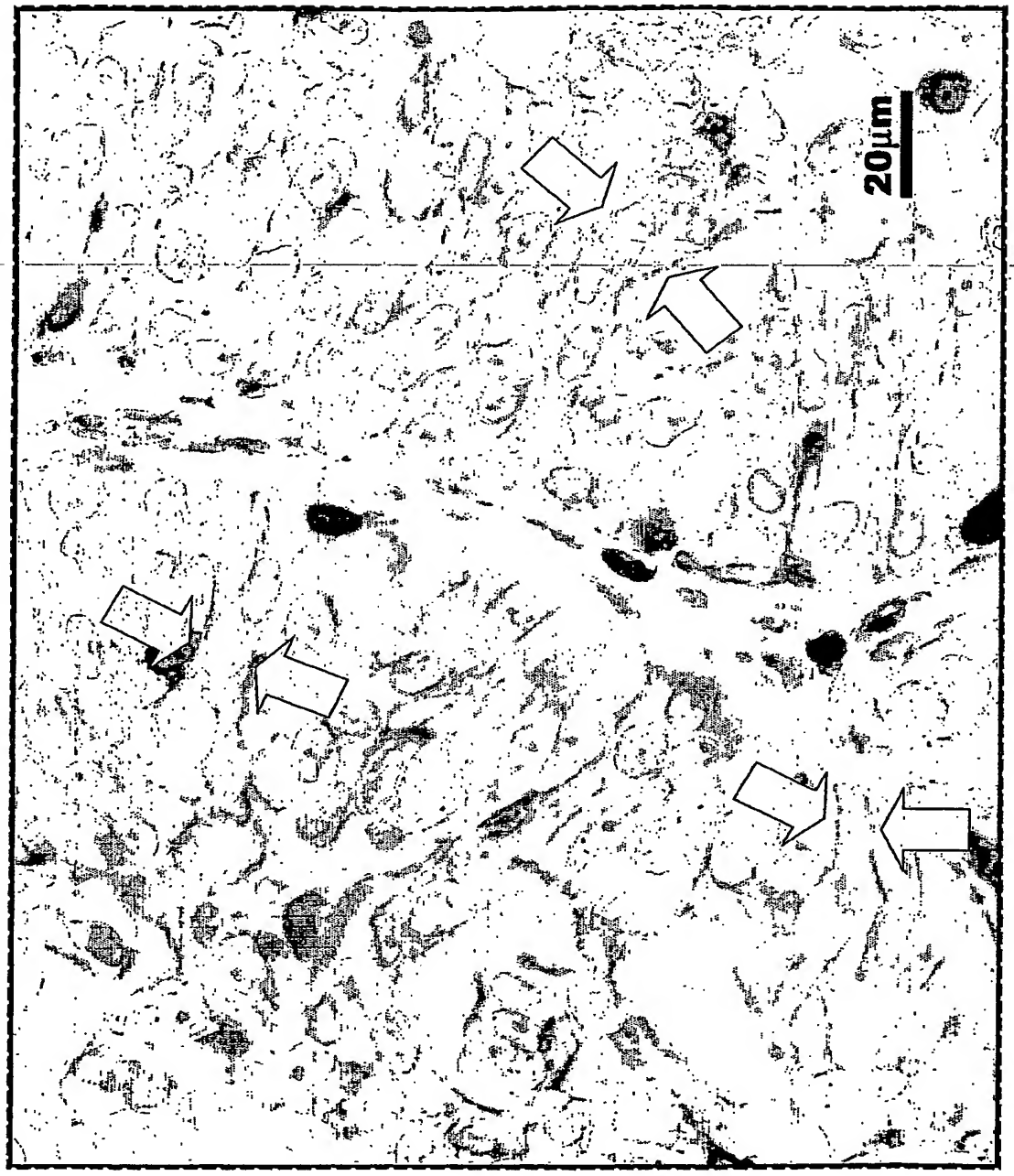


FIGURE 10. Pro104.D9 binds to Pro104 on the Cell Membrane of Ovarian Cancer Cells

FIGURE 11. Pro104.D133 binds to Pro104 on the Cell Membrane of Serous Ovarian Cancer Cells

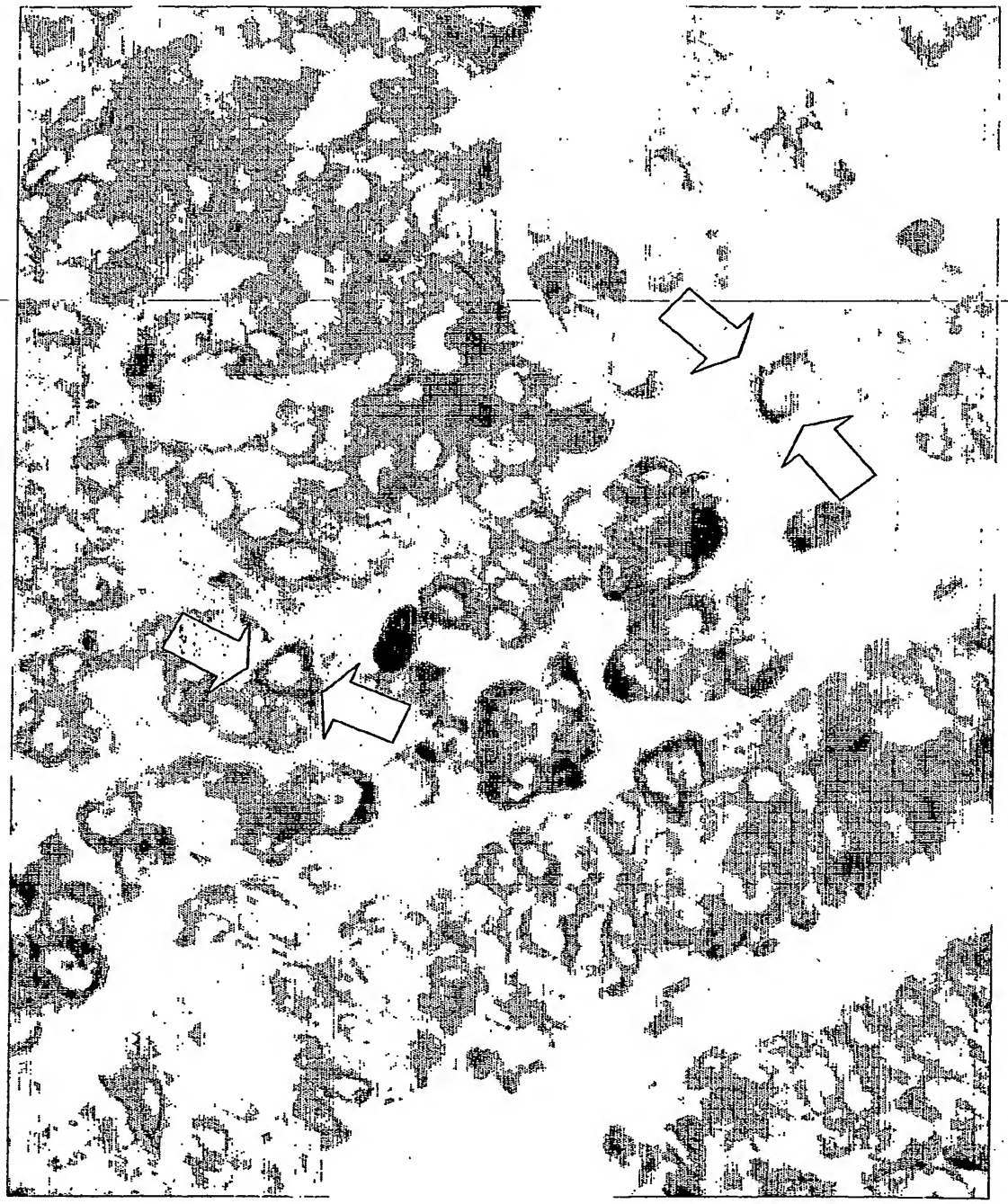


FIGURE 12. Pro104.C25.1 binds to Pro104 on Cancer Cells in Pancreatic Tumors

Fig.12A Pancreatic cancer 1

Fig. 12B Normal pancreas 1

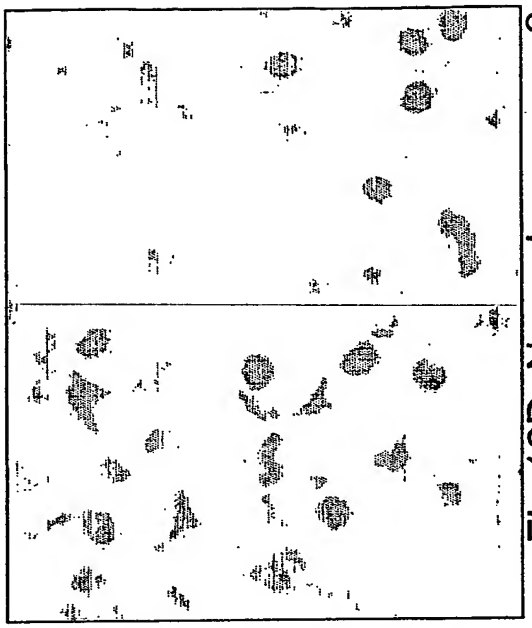


Fig.12C Pancreatic cancer 2

Fig.12D Normal pancreas 2

FIGURE 13. Controls Demonstrating Pro104 MAb Immunolabeling Specificity

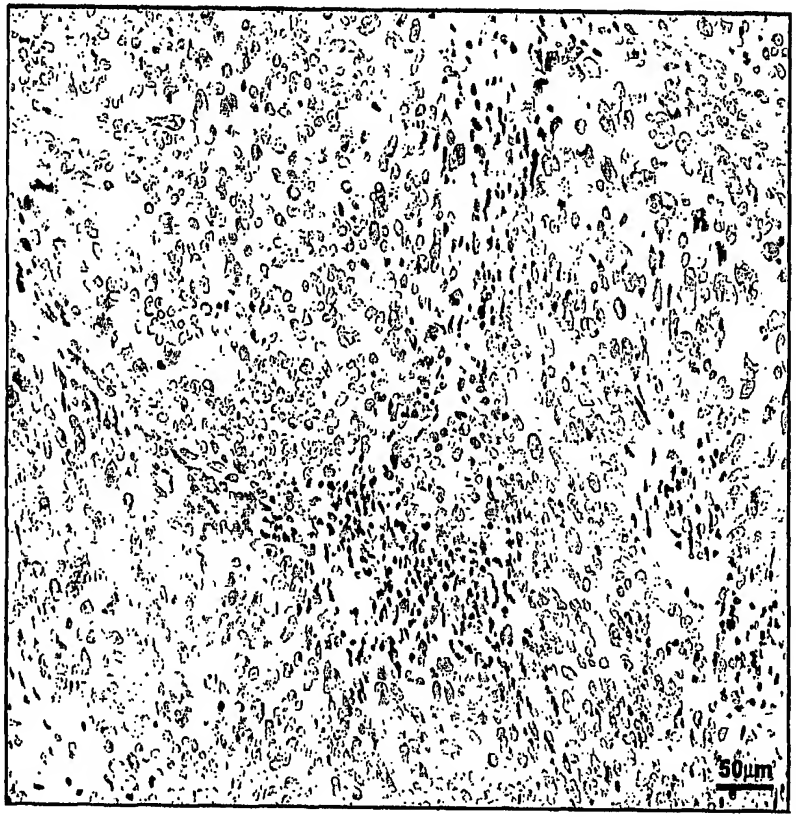


Fig. 13A Mouse IgG

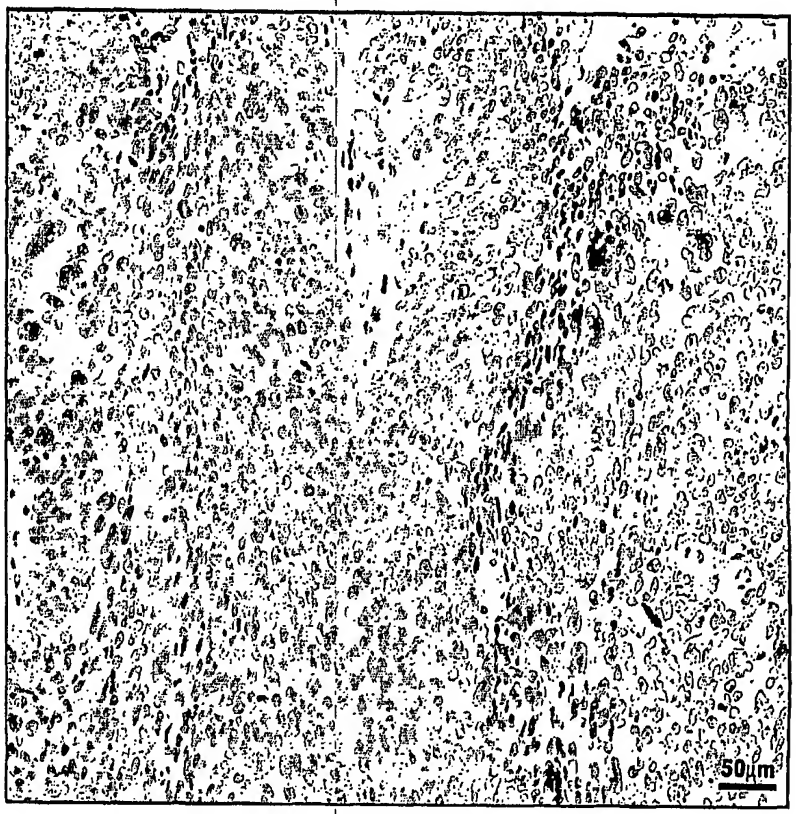
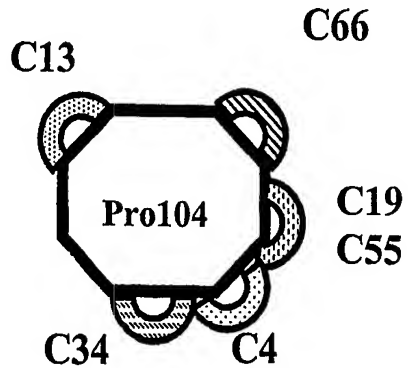


Fig. 13B Absorption with Pro104 Antigen

Figure 14: Epitope Map of Pro104 MAbs



Pairs tested on Training Serum and Cell Line Panel:
C4/ C13; C48/ C13; C13/ C18; C19/ C48; C55/ C34; C66/ C18; C19/C25; C55/C25

FIGURE 15

Western Blot Showing Detection of Pro104 Protein in mRNA+ Cell Lines and Ovarian Tumor Tissue (T) but not Normal Adjacent Tissue (N)

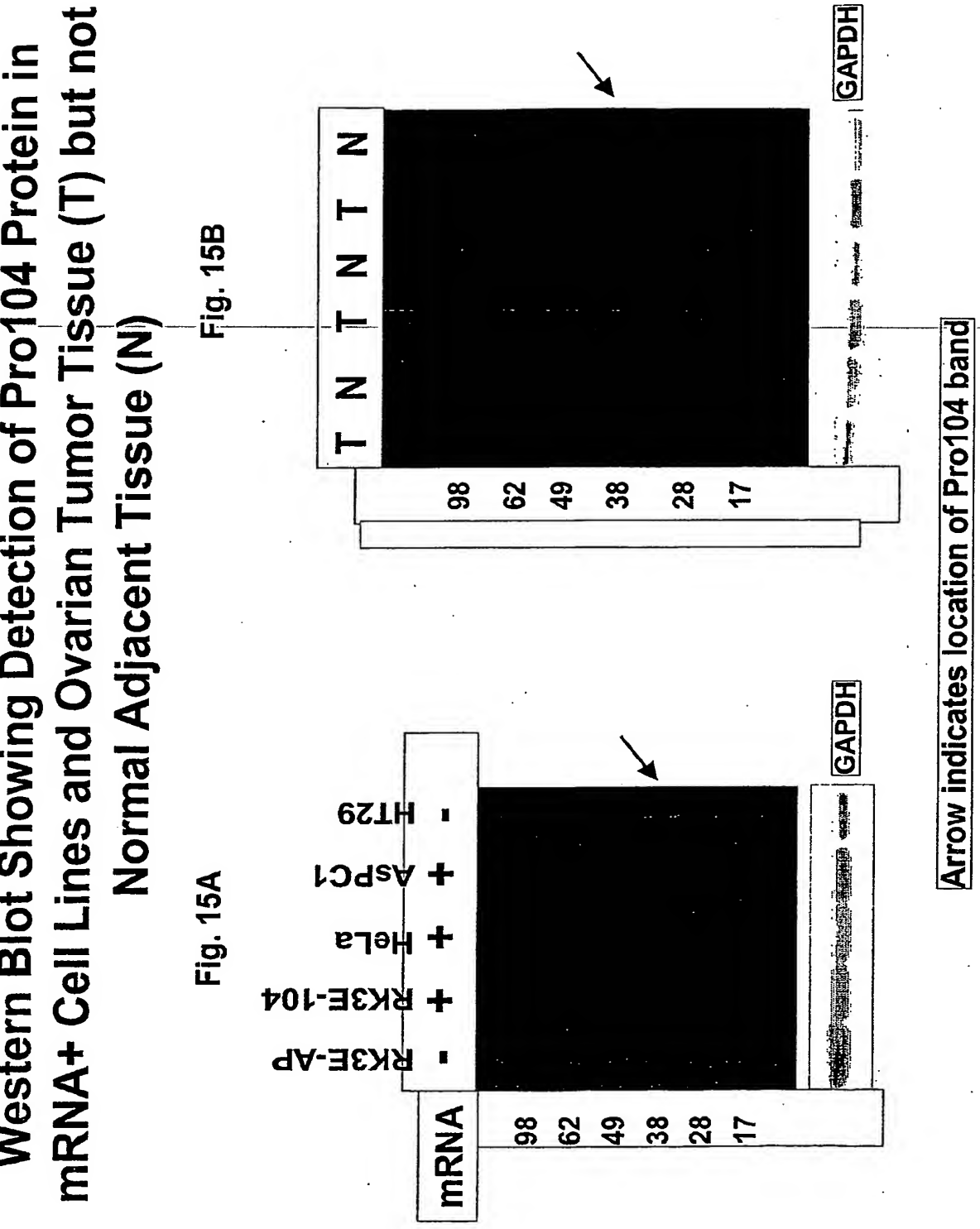


FIGURE 16

Overexpression of Pro104 Leads to Phosphorylation of EGF Receptor

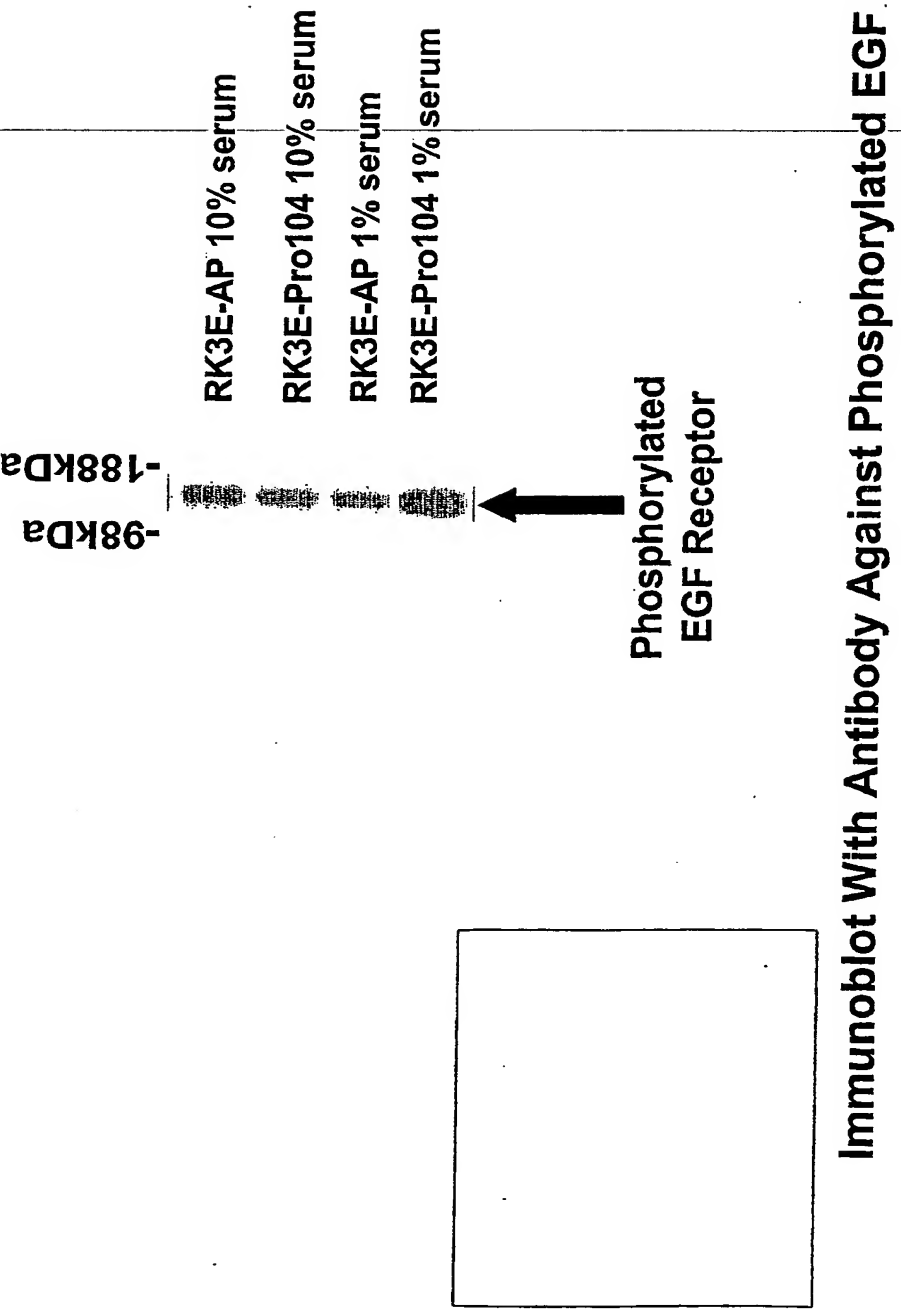


FIGURE 17
Pro104 Protein is Glycosylated and GPI-Linked

Fig. 16A

Native Pro104 is de-glycosylated in HeLa cell lines and ovarian tumors when treated with PNGaseF

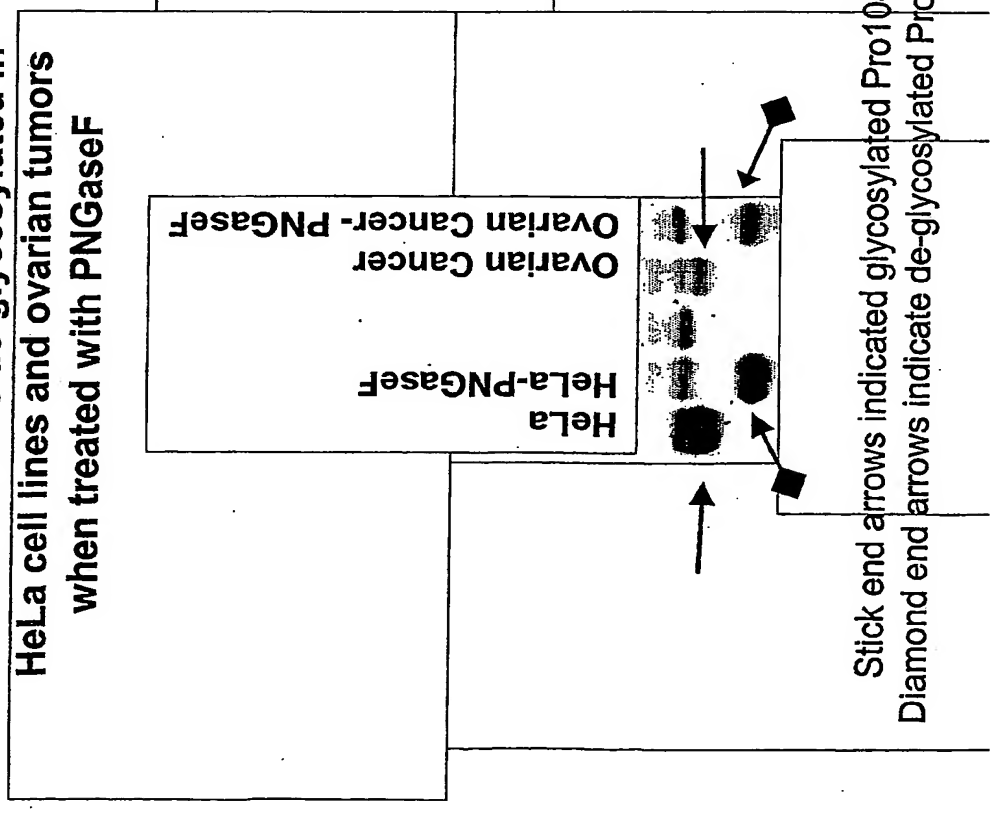


Fig. 16B

Native Pro104 shed into the media when treated with PI-PLC

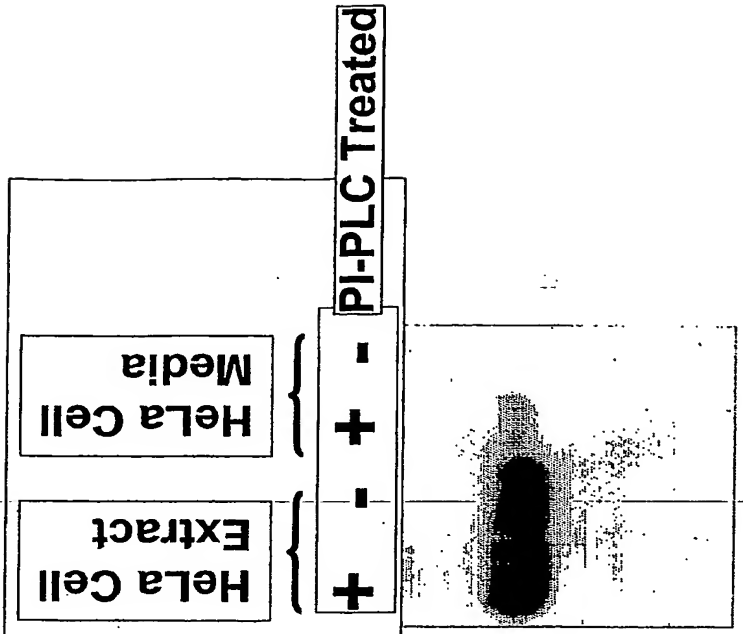


FIGURE 18

Surface Biotinylation of Native Pro104 in Cell Lines

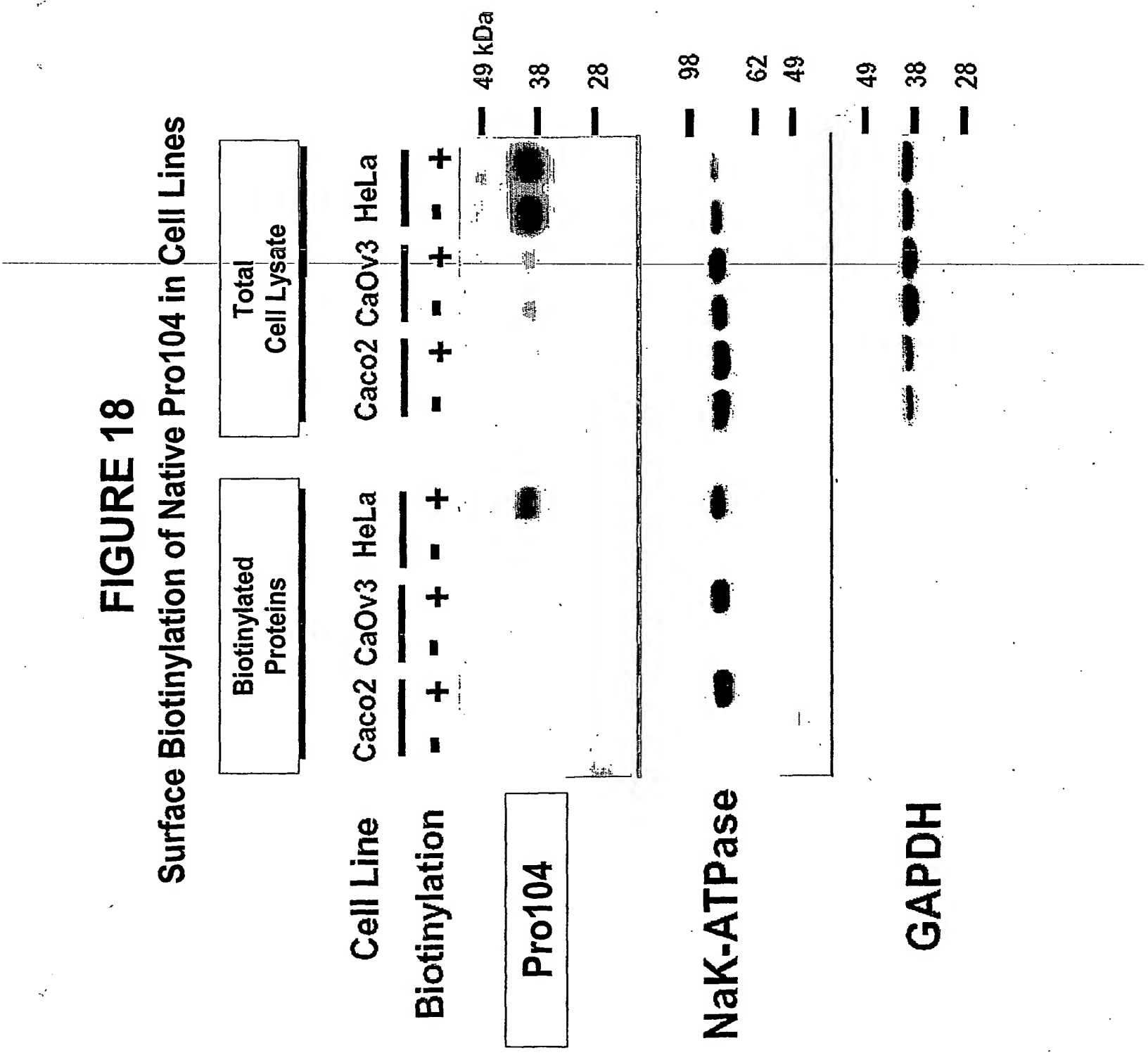
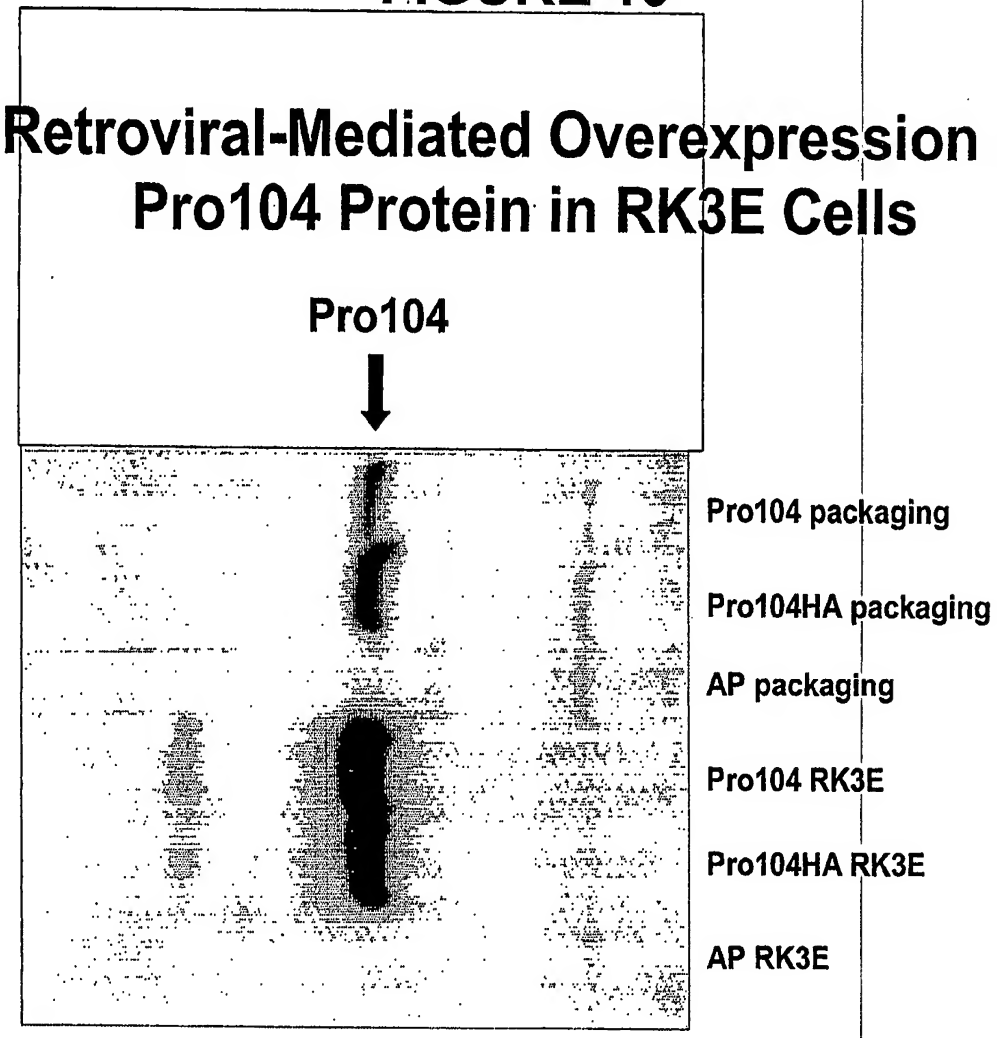


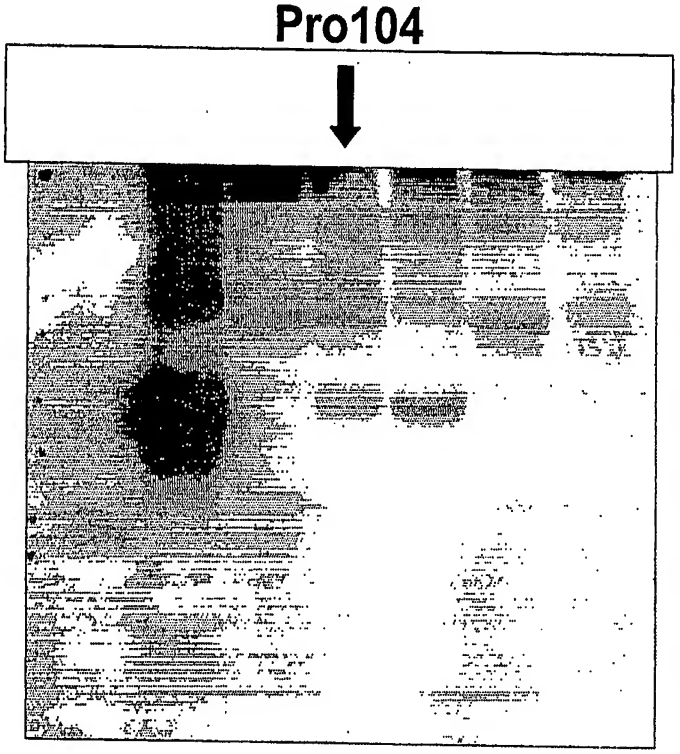
FIGURE 19



Western Immunoblot demonstrating Pro104 protein expression in retroviral packaging cell lines and virus infected RK3E cells

FIGURE 20

Retroviral-Mediated Overexpression of Pro104 Protein in SKOV3 Cells



Western Immunoblot demonstrating Pro104 protein expression in retroviral packaging cell lines and virus infected SKOV3 cells

FIGURE 21

siRNA Mediates Specific Down-Regulation of Pro104 Protein in HeLa Cells

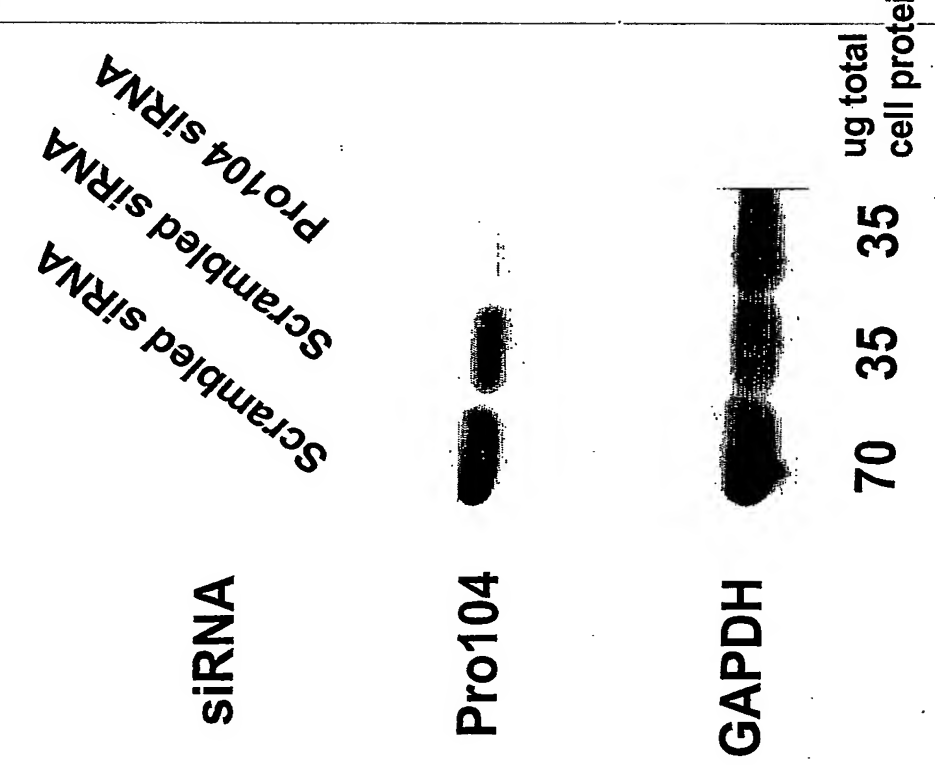


FIGURE 22

siRNA Mediates Down-Regulation of Pro104 Protein in CaOV3 Cells

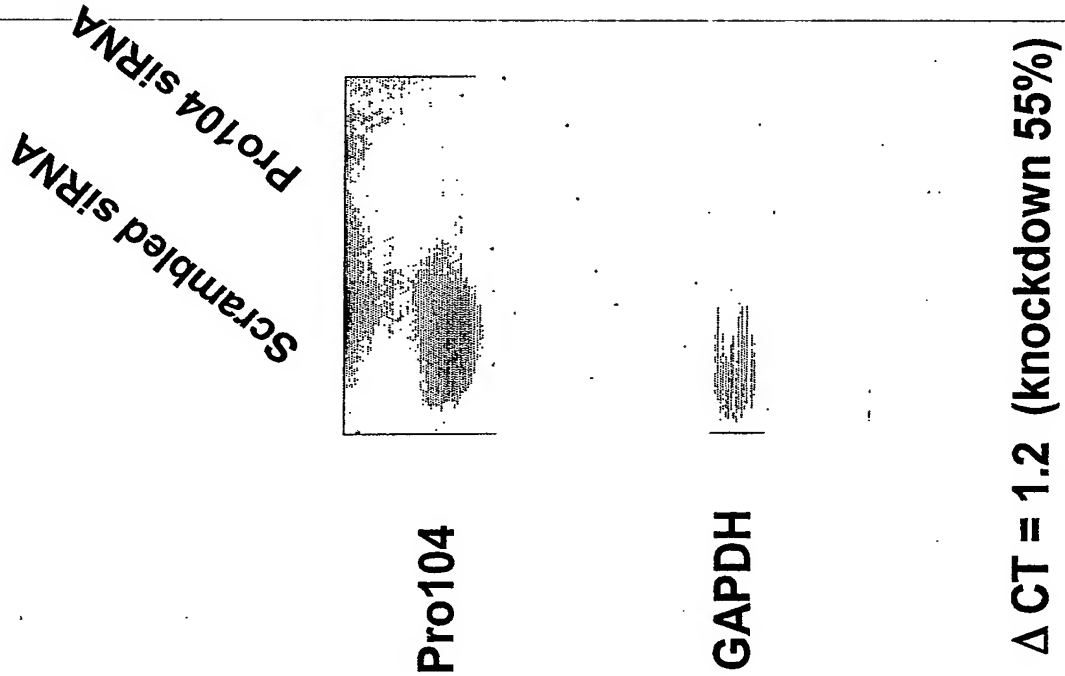
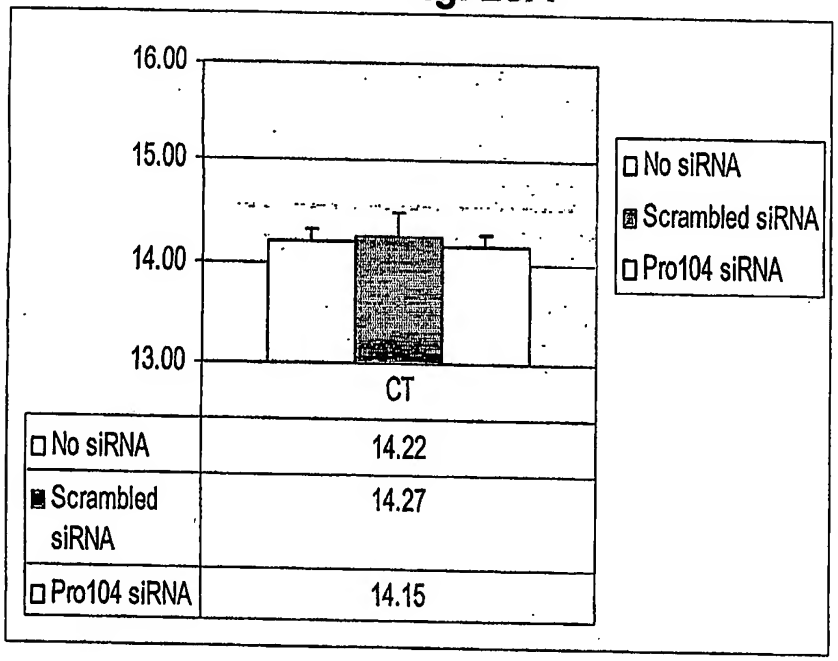


FIGURE 23

Pro104 siRNA Specifically Knockdown Pro104 mRNA in CaOV3 Cells

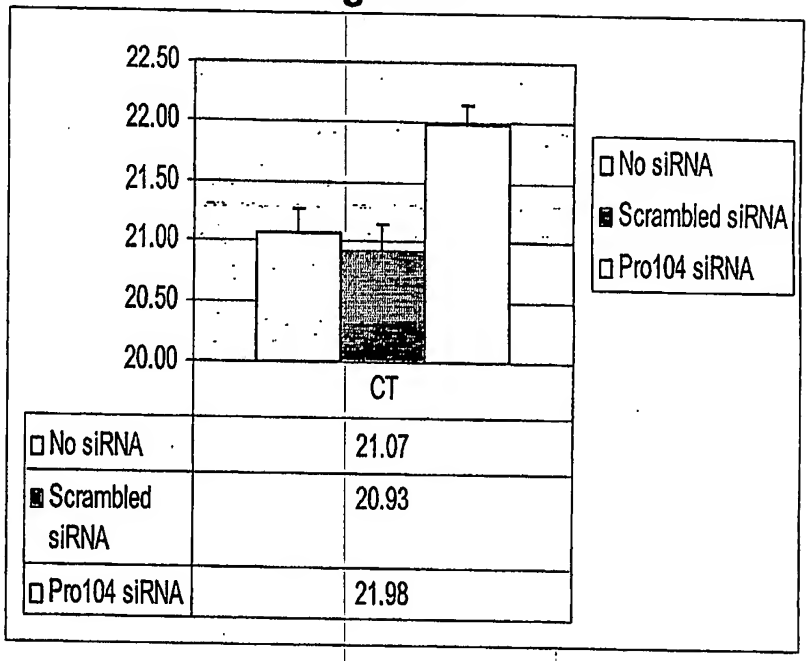
Fig. 23A



No siRNA	14.22
Scrambled siRNA	14.27
Pro104 siRNA	14.15

GAPDH Q-PCR Primers

Fig. 23B



No siRNA	21.07
Scrambled siRNA	20.93
Pro104 siRNA	21.98

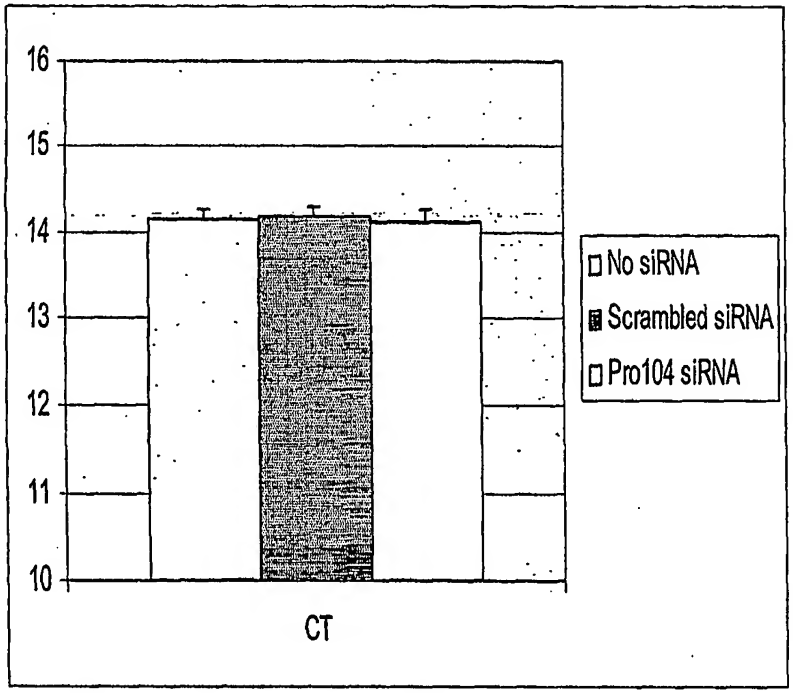
Pro104 Q-PCR Primers

Δ CT =1 for Pro104 (Knockdown 50%)

FIGURE 24

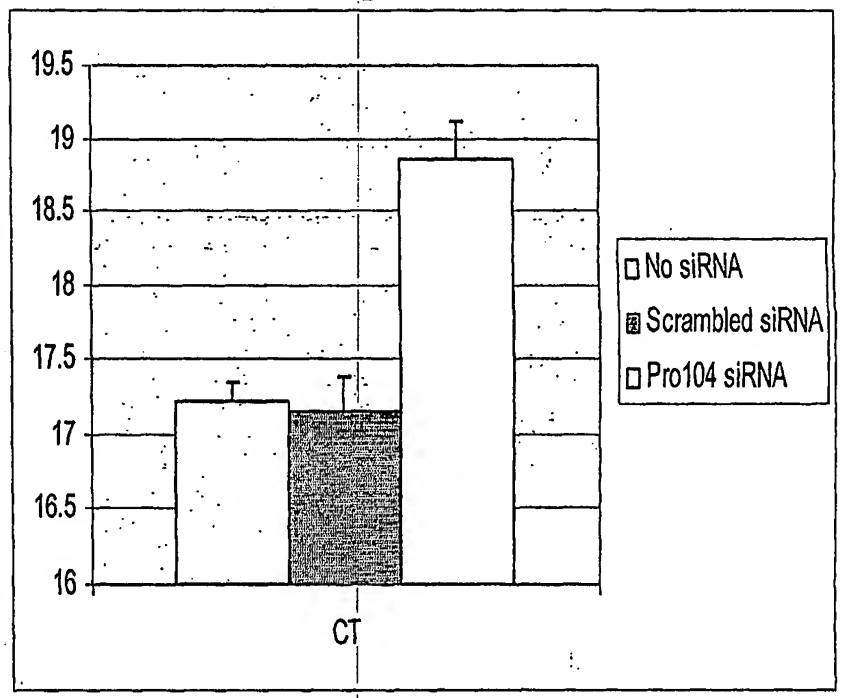
Pro104 siRNA Specifically Knockdown Pro104 mRNA in HeLa Cells

Fig. 24A



GAPDH Q-PCR Primers

Fig. 24B

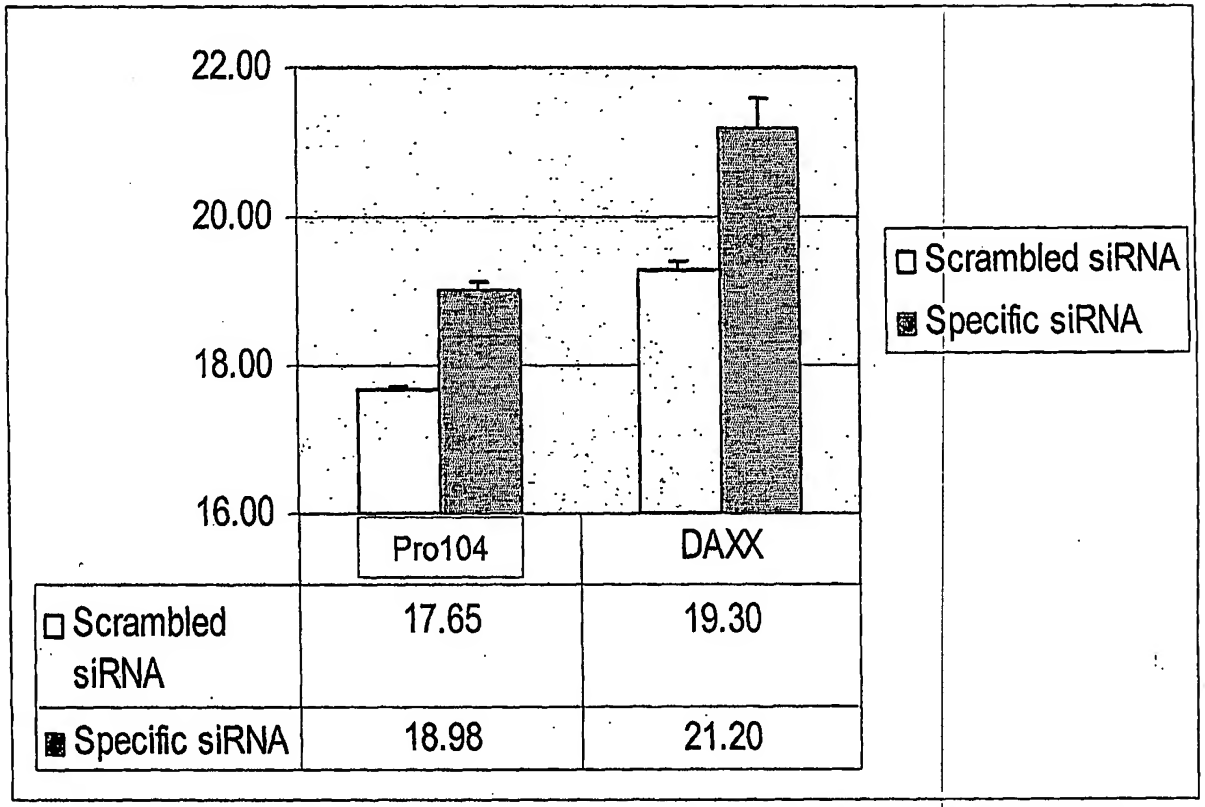


Pro104 Q-PCR Primers

Δ CT =2 for Pro104 (Knockdown 75%)

FIGURE 25

Pro104 siRNA Specifically Knockdown Pro104 mRNA in HeLa Cells, Compared to a Positive Control



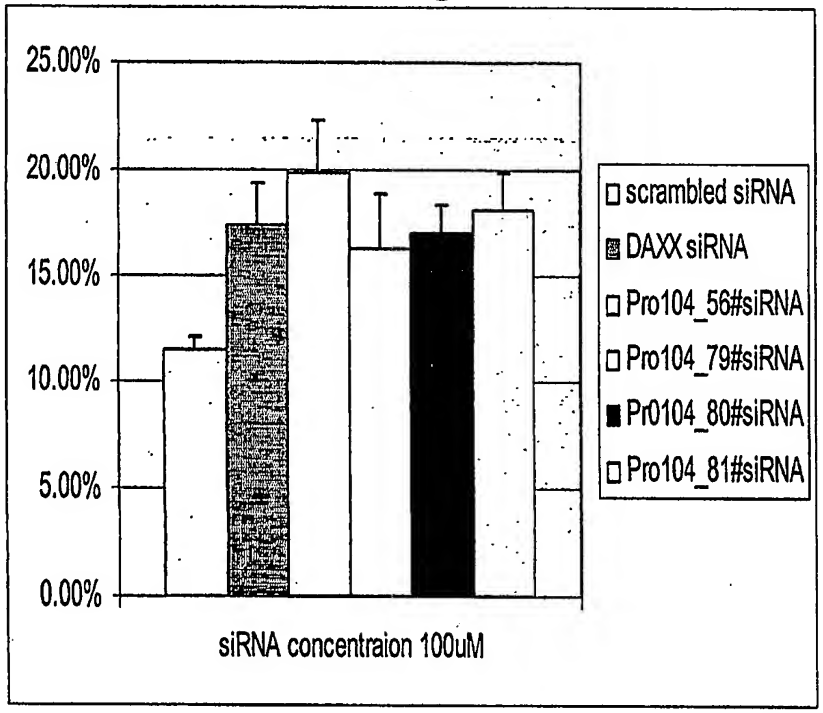
	Pro104	DAXX
Scrambled siRNA	17.65	19.30
Specific siRNA	18.98	21.20

QPCR with specific primers for Pro104 and DAXX (positive control for apoptosis) demonstrates mRNA knockdown

FIGURE 26

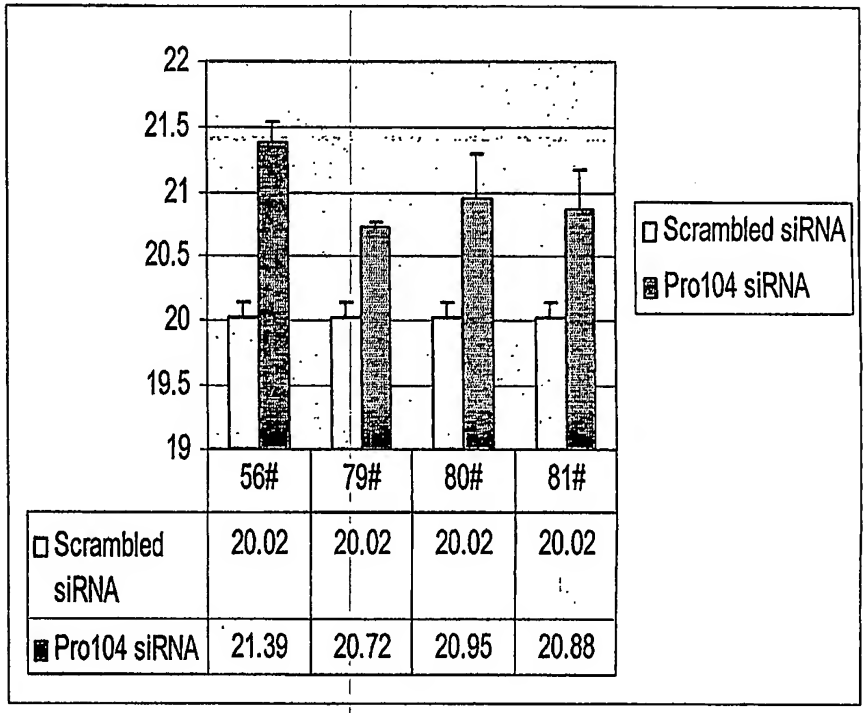
Different Pro104 siRNAs Induce Specific mRNA Knockdown and Apoptosis in HeLa Cells

Fig. 26A



Annexin V assay for apoptosis
(DAXX positive control)

Fig. 26B



	56#	79#	80#	81#
Scrambled siRNA	20.02	20.02	20.02	20.02
Pro104 siRNA	21.39	20.72	20.95	20.88

QPCR demonstrates Pro104 mRNA knockdown

FIGURE 27

Specific Knockdown of Pro104 mRNA in HeLa Cells Induces Cell Death

Fig. 27A

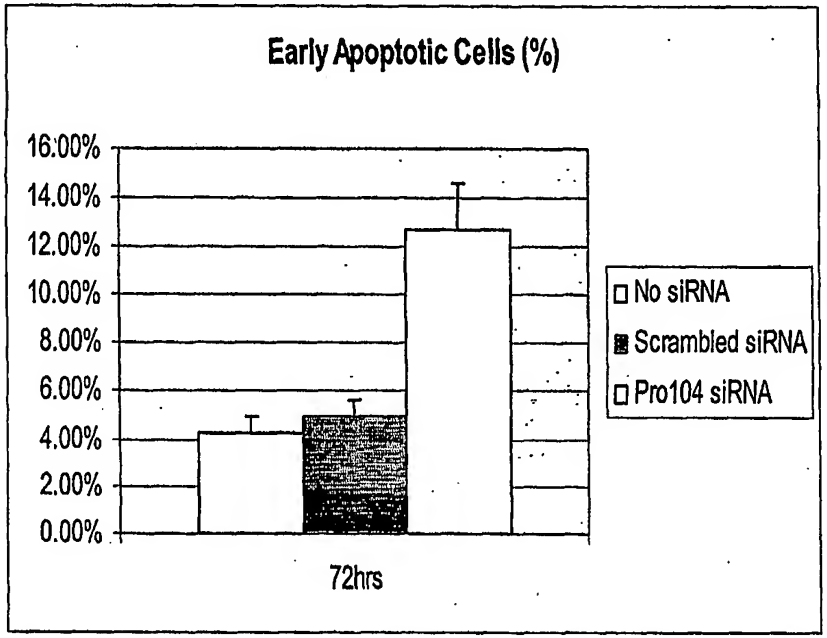
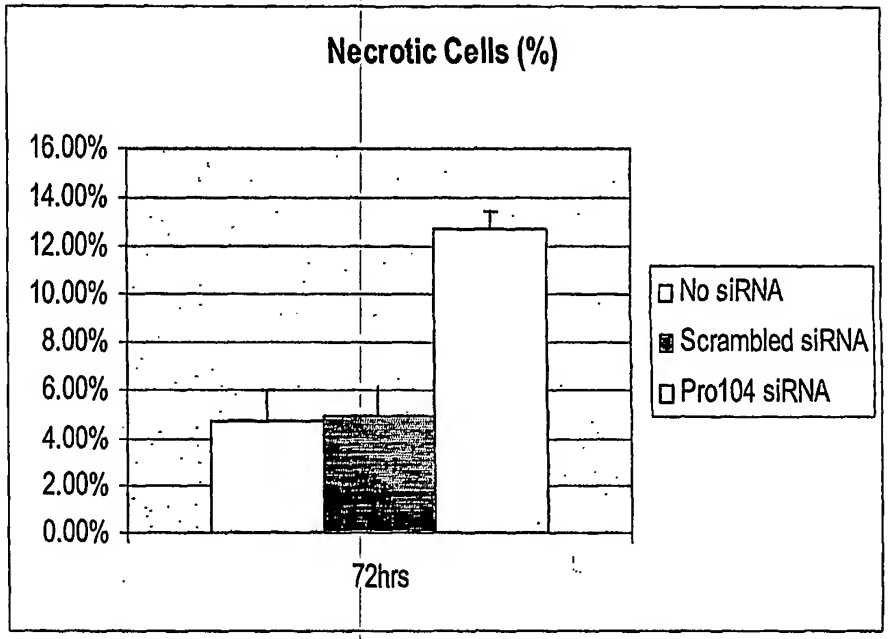


Fig. 27B



Annexin V Assay

FIGURE 28

Specific mRNA Knockdown by Pro104 siRNA Induces Apoptosis in HeLa Cells; Measured by Two Different Methods

Fig. 28A

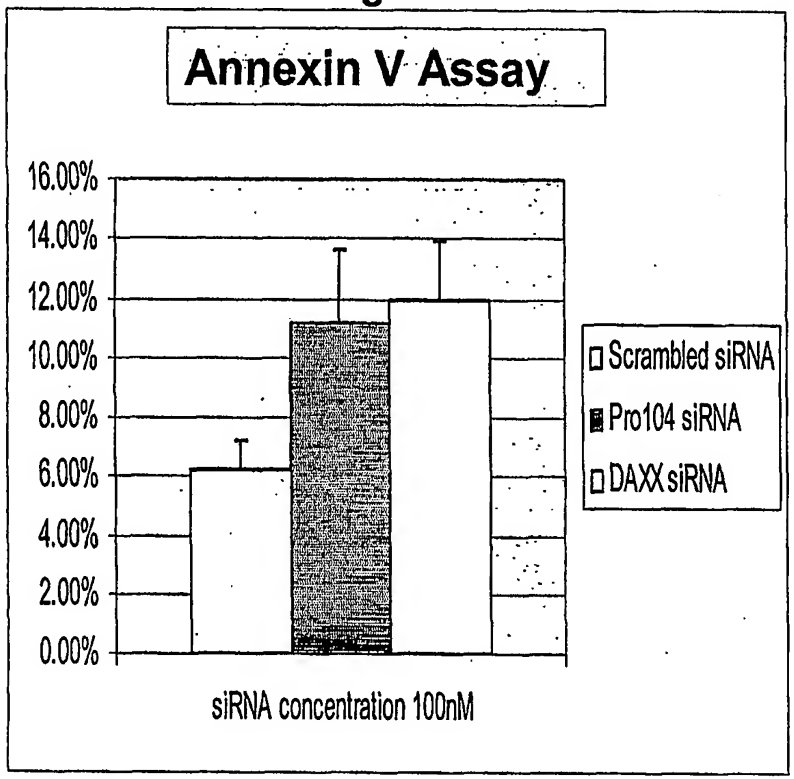
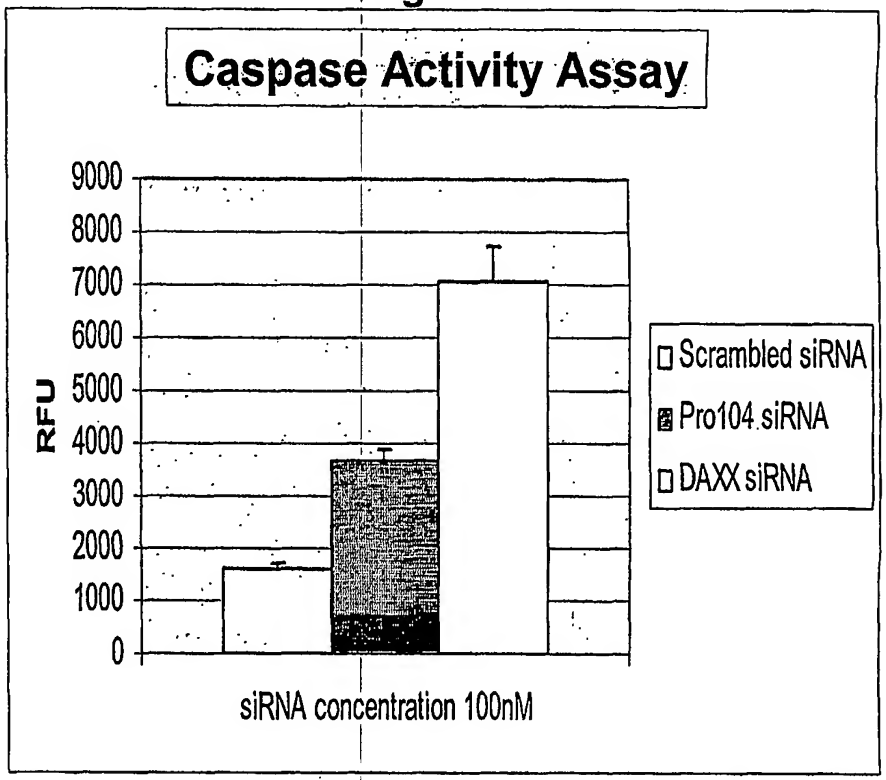


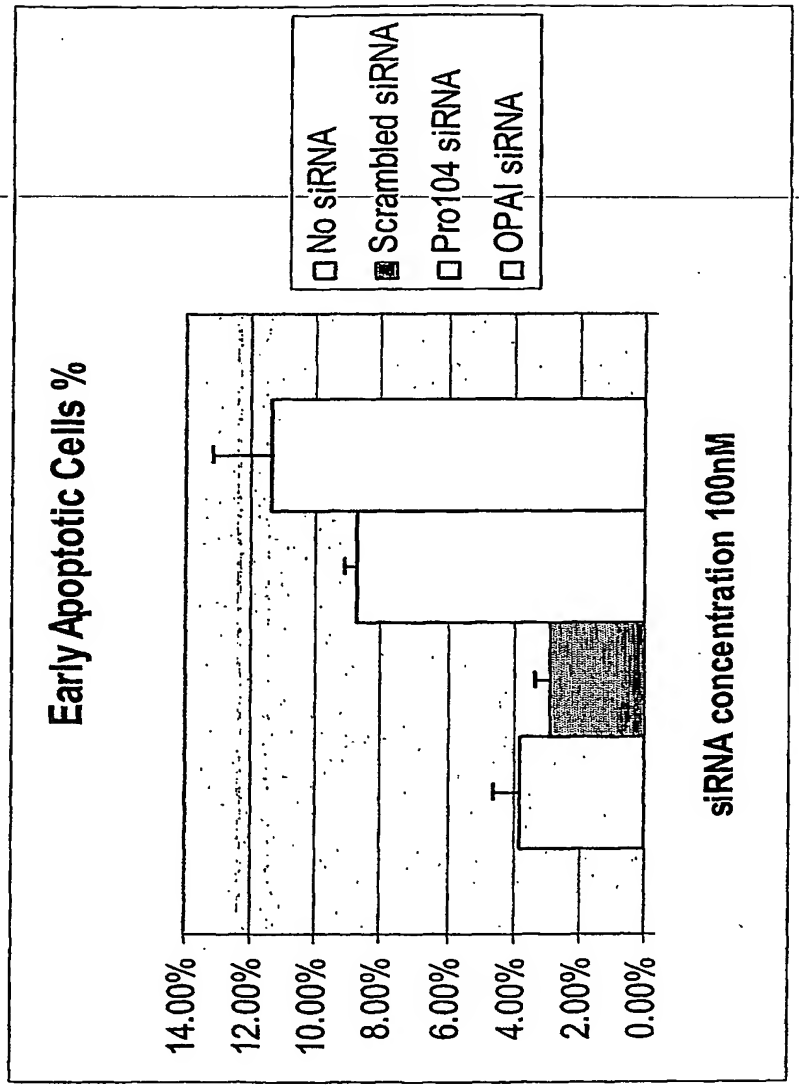
Fig. 29B



Apoptosis assays of cells treated with Pro104 siRNA

FIGURE 29

Specific Knockdown of Pro104 mRNA in CaOV3 Cells Induces Apoptosis

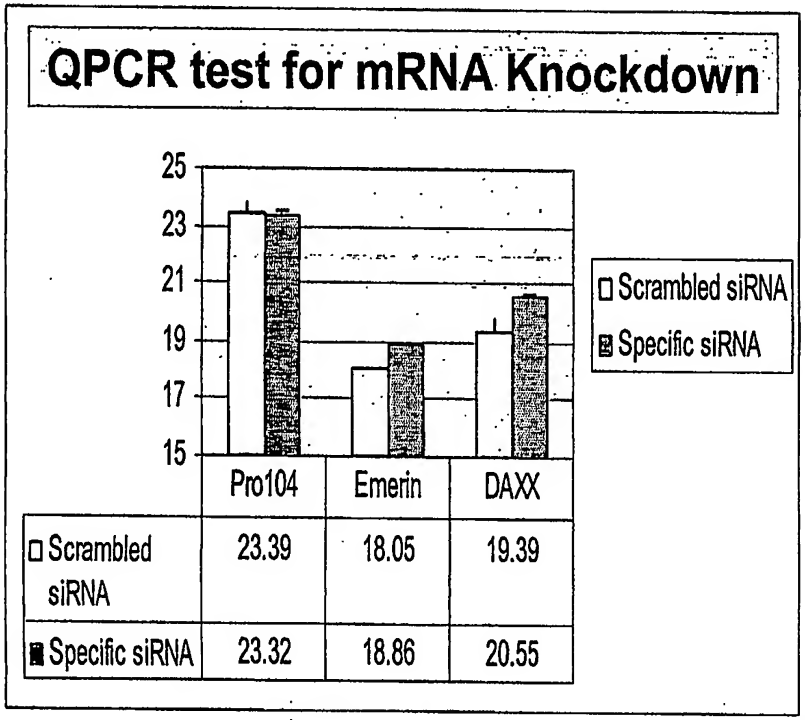


Annexin V Assay

FIGURE 30

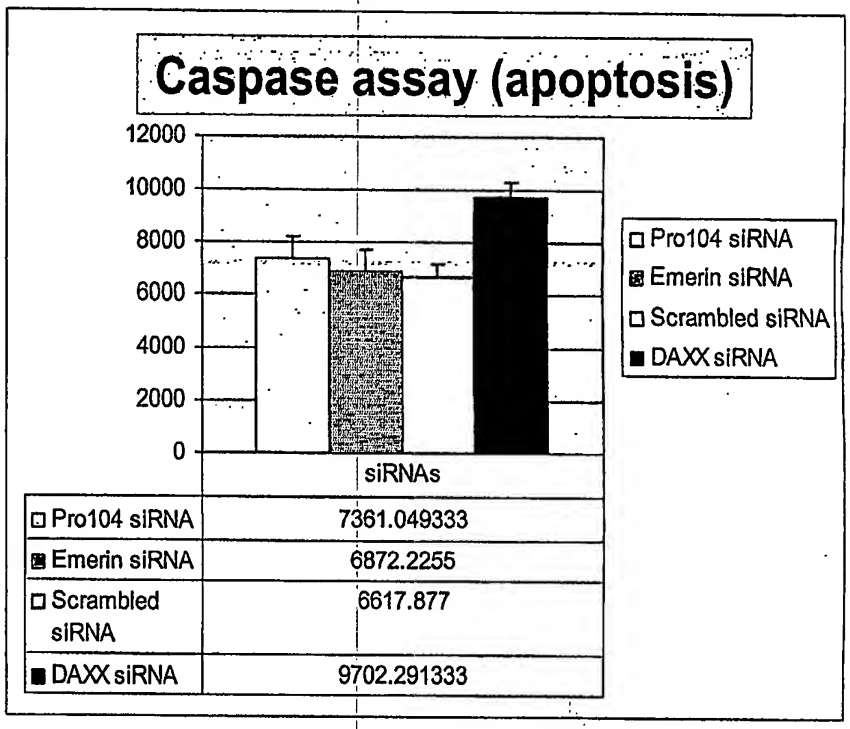
Pro104 siRNA Has no Effect on Apoptosis in Cells Without Pro104 mRNA

Fig. 30A



	Pro104	Emerin	DAXX
Scrambled siRNA	23.39	18.05	19.39
Specific siRNA	23.32	18.86	20.55

Fig. 30B



Pro104 siRNA	7361.049333
Emerin siRNA	6872.2255
Scrambled siRNA	6617.877
DAXX siRNA	9702.291333

SKBR3 cells

Knockdown:

Pro104:none (no mRNA)

DAXX: 65% (positive control)

Emerin: 50% (mRNA +, non-essential)

FIGURE 31

Overexpression of Pro104 Induces Cell Growth in Soft Agar

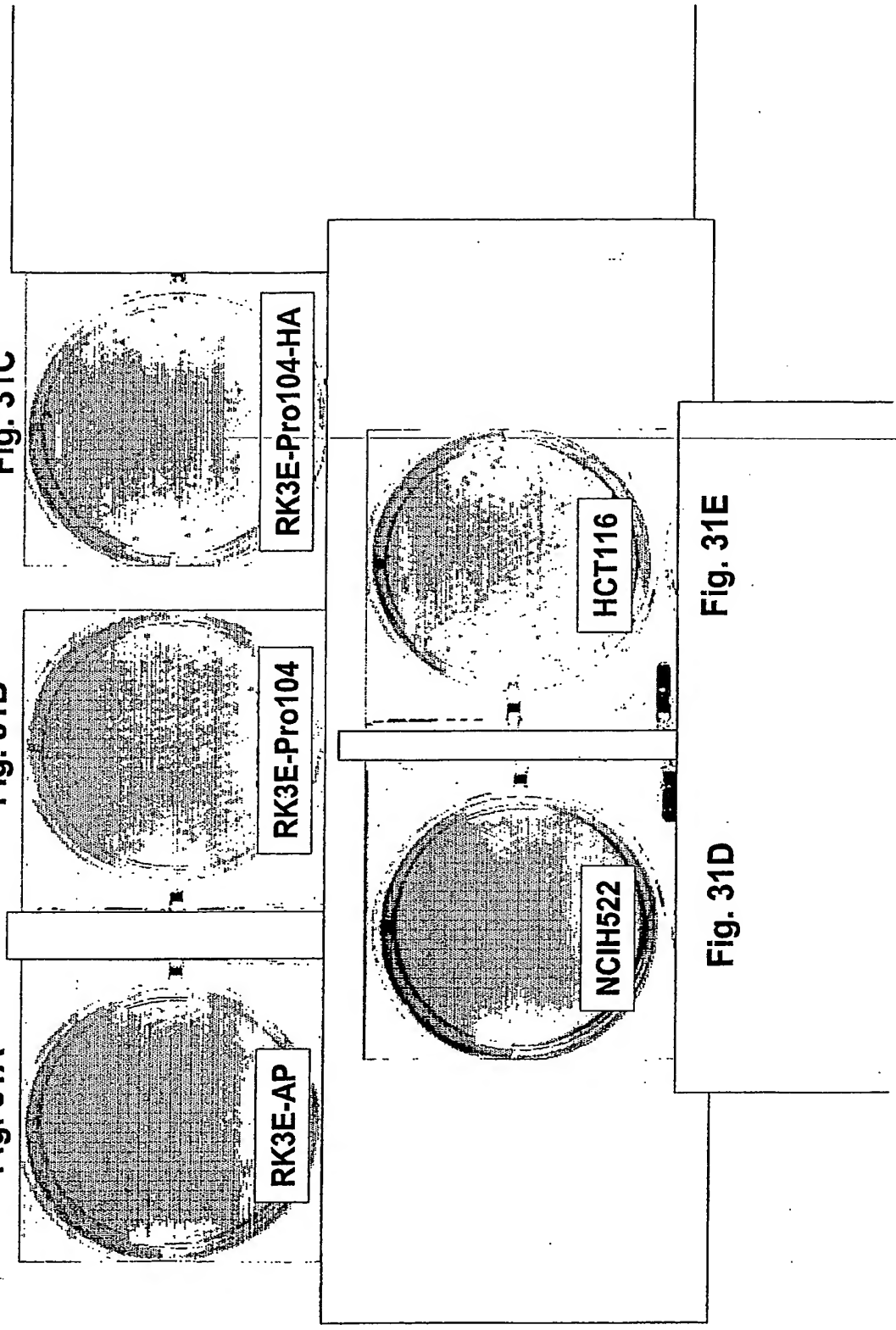


FIGURE 32
Pro104 Protease Activity is Required for Cell Growth

Fig. 32A

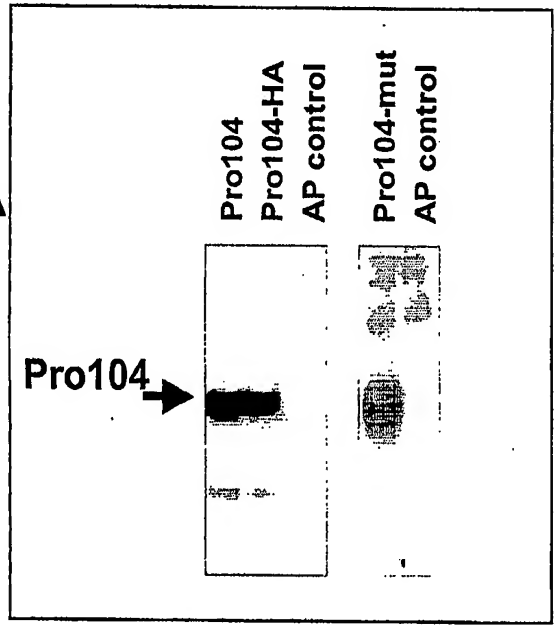


Fig. 32C

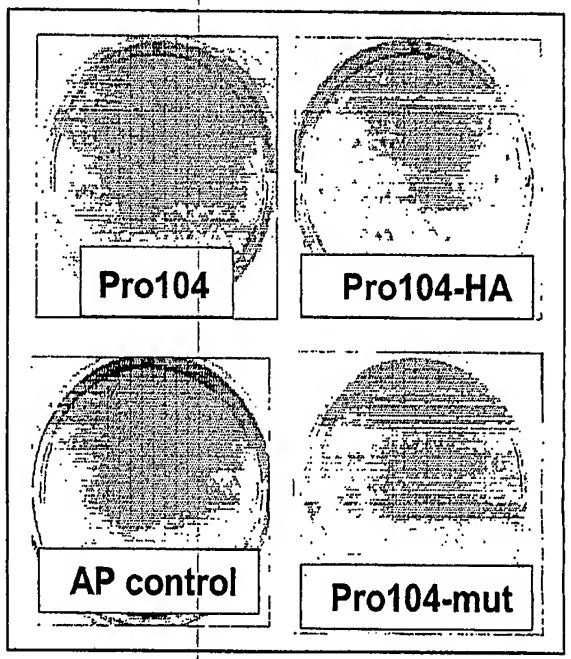


Fig. 32B

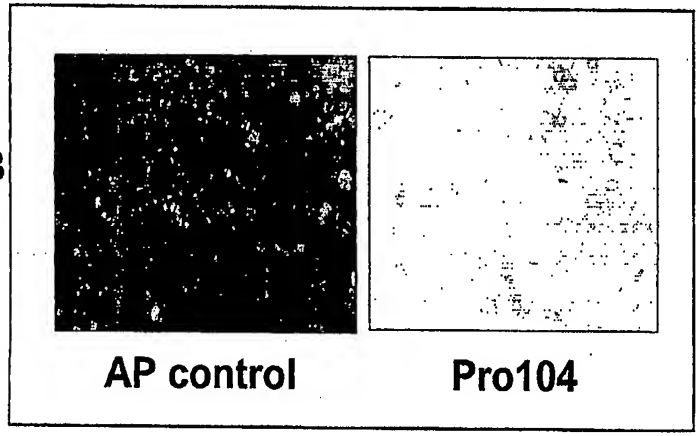


Fig. 32D

Cell Type	# Colonies per field
AP control	0
Pro104	60
Pro104-HA	68
Pro104-mut	0

RK3E cells with Pro104-mut lack Pro104 protease activity

FIGURE 33
Knockdown of Pro104 mRNA by siRNA Inhibits Growth of HeLa Cells in Soft Agar

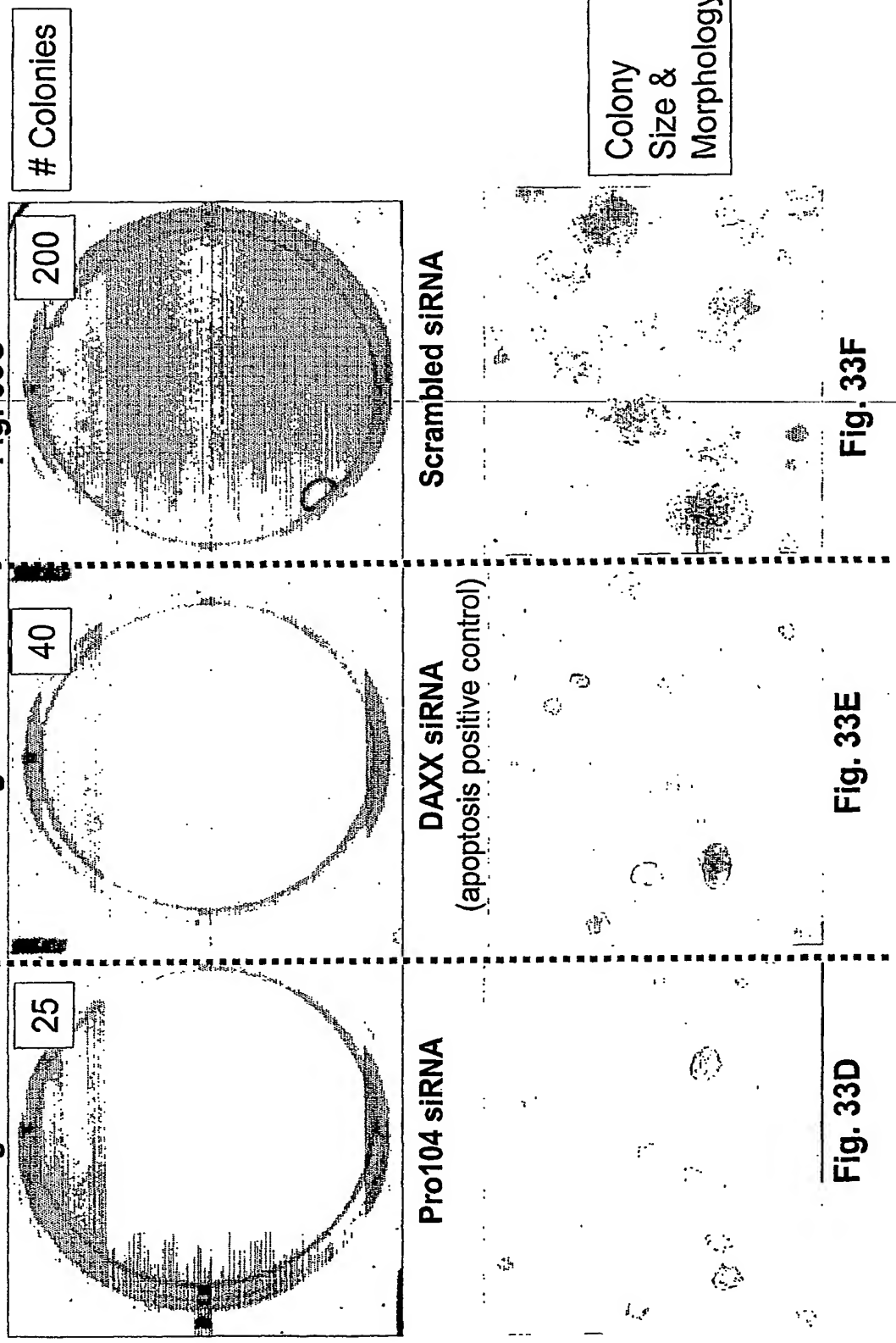


FIGURE 34

Knockdown of Pro104 mRNA by siRNA Inhibits Growth of HeLa Cells in Soft Agar

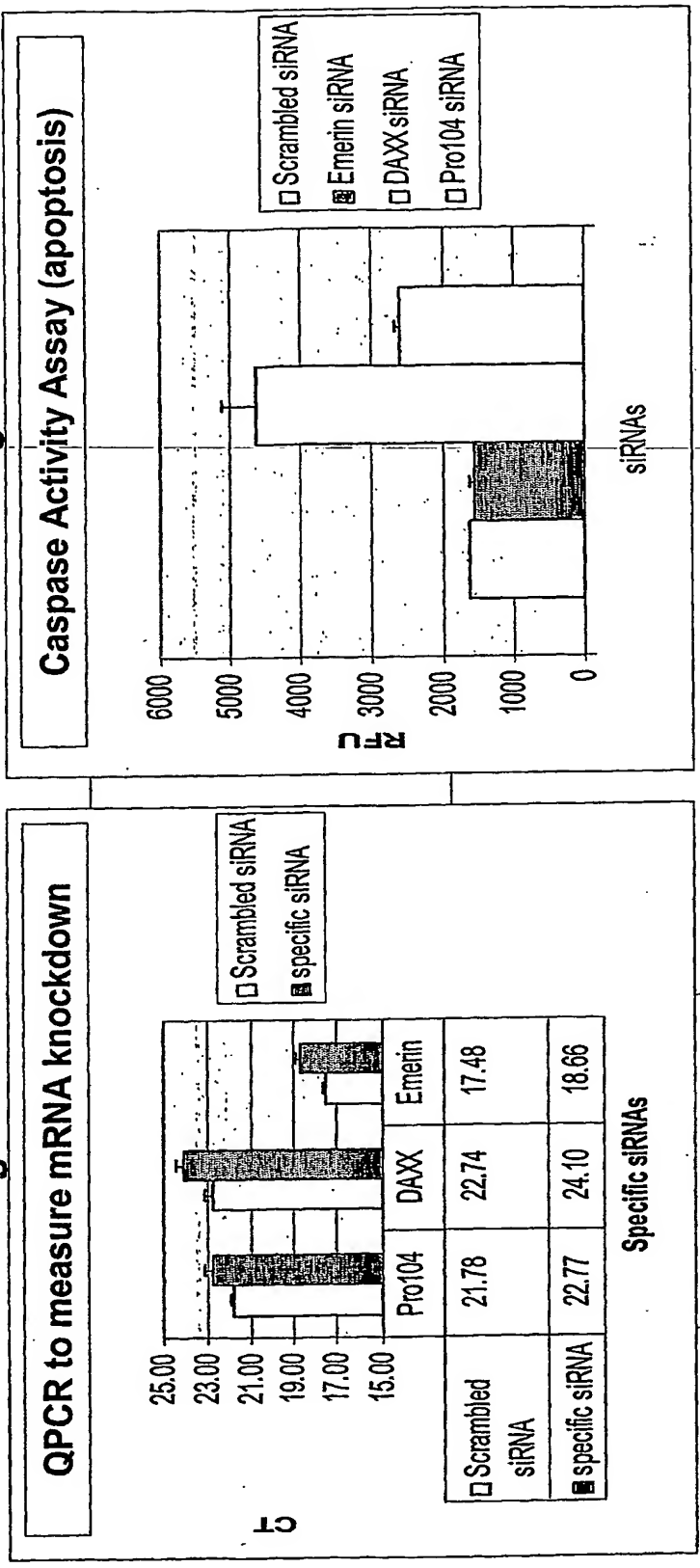


FIGURE 35

Increased Growth of Human Tumor Cells Over-Expressing Pro104

Fig. 35A

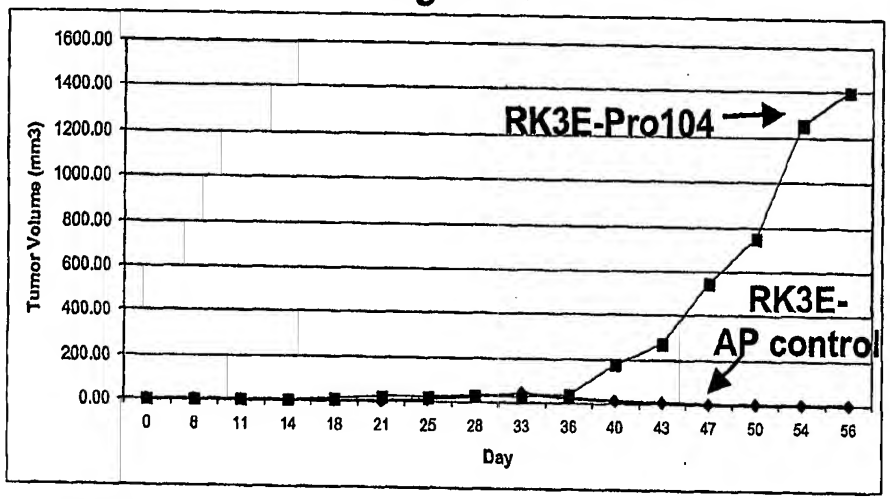


Fig. 35B

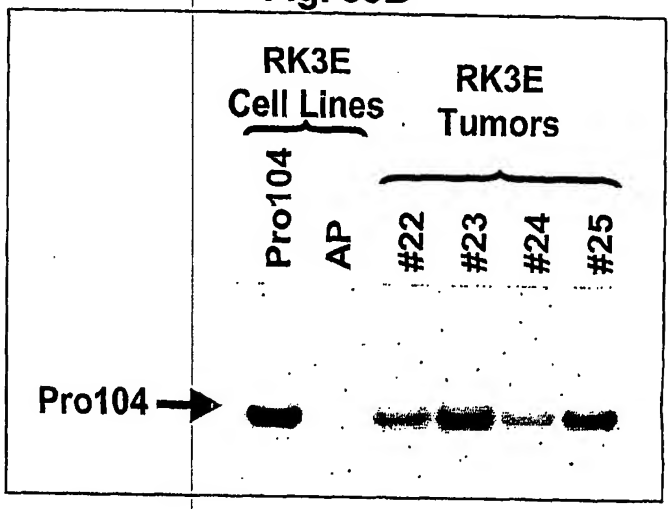


Fig. 35C

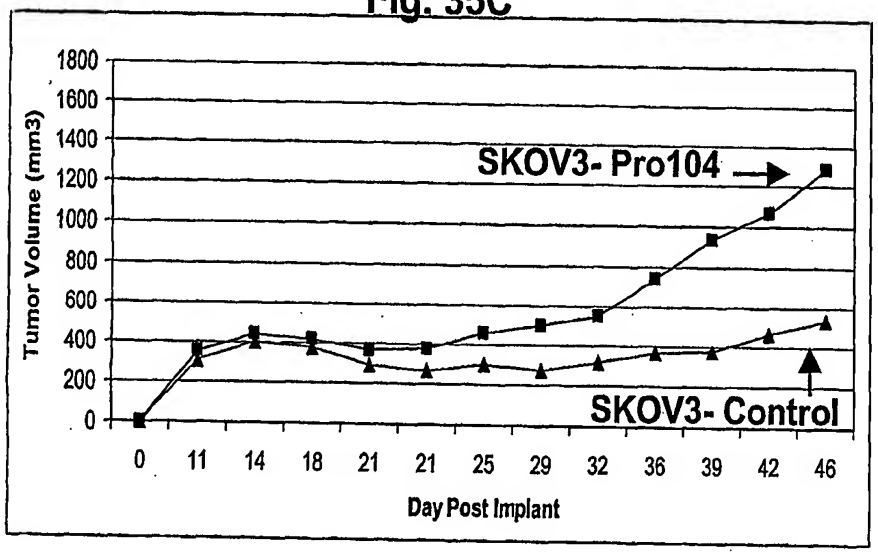


Fig. 35D